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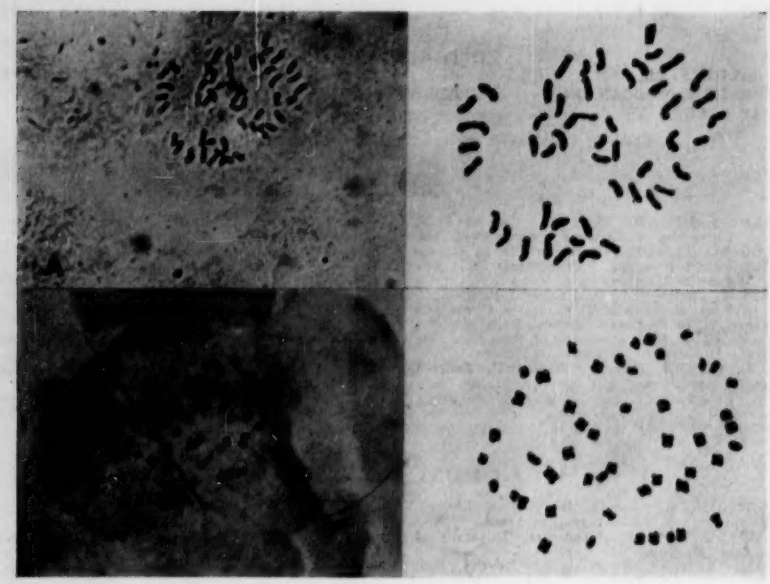

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Crossbred Dwarf Cattle

Extreme Non-Agouti Mice

Muscle Abnormality in the Fowl

Flower Color Inheritance in *Mimulus*

Reanalysis of the Male Tortoiseshell Cat

VOLUME 47 SEPTEMBER-OCTOBER, 1956 NUMBER 5

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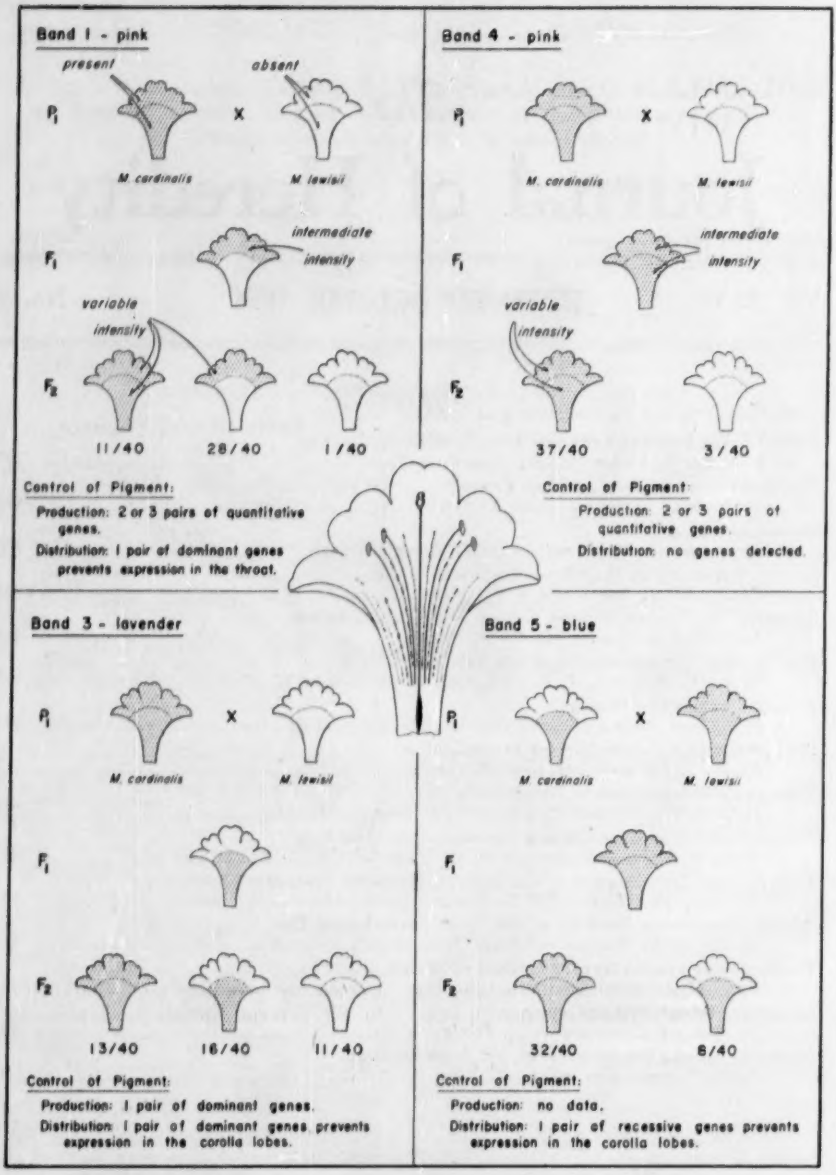

PRODUCTION AND DISTRIBUTION OF ANTHOCYANIN PIGMENT

Figure 1

The distribution of each pigment is indicated by shading of the appropriate areas of the corolla diagrams. Some pigments are found in the whole corolla whereas others are limited to the corolla lobes or corolla throats. The dotted lines indicate the division between these two areas. Note that each pigment exhibits a different mode of inheritance, except #6 (not shown) which is similar to, but independent of, #5. Band #2 was omitted for lack of data.

FLOWER COLOR INHERITANCE IN THE MIMULUS CARDINALIS COMPLEX

ROBERT K. VICKERY, JR. AND RICHARD L. OLSON*

THIS investigation is an outgrowth of a study of intersectional crossing barriers in the genus *Mimulus*[2]. In that experiment red-flowered *Mimulus cardinalis* Dougl. was found to hybridize with lavender-flowered *M. lewisii* Pursh. Whereas the F_1 was uniformly pink flowered, the F_2, consisting of 1,000 individuals, exhibited an array of flower colors from deep red to light lavender with no two plants having flowers of exactly the same hue. This range of variability among the F_2 was so striking that we were prompted to investigate its genetic basis.

On further study the flower colors of the parental species were found to vary from one population to another. *M. cardinalis* displays different shades of red and orange, whereas *M. lewisii* exhibits various tints of lavender, pink, and magenta. With this natural interpopulation variability in mind, the study was extended by including as many different populations, races, and species of the *M. cardinalis* complex as could be obtained (see Table I).

The large number of phenotypes observed in the various populations, races, and hybrids, particularly in the F_2 populations, indicate a complex genetic situation. Therefore, in order to simplify the analysis, we isolated the component flower pigments of each race or population and then studied their distribution in the F_1 and F_2 hybrids in order to determine the mode of inheritance of each pigment. Following techniques suggested by Dr. John D. Spikes of the Department of Experimental Biology, University of Utah, we successfully extracted the following classes of pigments: carotenoids, xanthophylls, anthoxanthins, and anthocyanins. The extracts were then chromatogrammed in order to separate the component pigments of each class.

Extraction of Pigments

The carotenoid pigments were extracted by grinding the dried corollas in petroleum ether. After centrifugation, the supernatant liquid was chromatogrammed using 2×27 cm. strips of Whatman #1 filter paper. The liquid was spotted 2.0 cm. from the bottom of the strips. When dry, the strips were suspended in test tubes so that the bottom 0.5 cm. was immersed in a solvent mixture consisting of 10 percent acetone and 90 percent petroleum ether. The solvent advanced rapidly up the filter paper carrying the pigment with it. After approximately 15 minutes, the strips were removed from the test tubes and dried. Careful inspection of the chromatograms revealed only one carotenoid pigment band. It was present in all the populations, races, and hybrids and had an R_f† value of 1.0.

The xanthophyll pigments were extracted by grinding the dried corollas in 85 percent methanol. Upon chromatogramming the supernatant liquid with a solvent mixture consisting of 50 percent acetone and 50 percent petroleum ether, a single xanthophyll pigment was found. It was present in all of the plants and had an R_f value of 1.0.

* From the Department of Genetics, University of Utah, Salt Lake City 12, Utah. Approximately half of the work reported here was carried out by the junior author and was submitted by him to the faculty of the University of Utah in partial fulfillment of the requirements for the Master of Science degree. The authors thank Drs. G. Lefevre, Jr. and W. W. Newby of the Department of Genetics, University of Utah, for their many helpful suggestions during the investigation and for their reading of the manuscript. This work was supported by the University of Utah Research Fund.

† R_f = distance traveled by the pigment / distance traveled by the solvent.

Anthoxanthins may be distinguished from anthocyanins on the basis of color; the former are yellow, whereas the latter vary from red to blue. On this basis two anthoxanthins of $R_f = 1.0$ and $R_f = 0.75$ were detected. They were present in all of the races and hybrids. In addition, the chromatograms showed the presence of at least six different anthocyanin pigments. They were found to be distributed in a variety of combinations in the different races and their hybrids, (see Table I).

Discussion of the Yellow Pigments

In the *M. cardinalis* complex, the paper chromatographic separation of the individual pigments of each class revealed the presence of 10 flower color pigments: one carotenoid, one xanthophyll, two anthoxanthins, and six anthocyanins. The most outstanding fact brought out by the analysis of the pigment distribution was that the four yellow pigments (the carotenoid, the xanthophyll, and the two anthoxanthins) are present in all of the populations, races, and hybrids examined. On first thought, this result would seem to indicate that all of the plants are homozygous for the genes controlling the yellow pigments. However, a closer inspection of the chromatograms showed significant differences in the intensities of the yellow pigment bands. For example, the carotenoid band from *M. cardinalis* is strong, but that from *M. lewisii* is weak. The F_1 hybrids of the cross and three-fourths of the F_2 population also have weak carotenoid bands. A microscopic examination of the flowers showed that all of the plants which register a weak carotenoid band have carotenoid granules only in the corolla hairs, whereas the plants which show a strong carotenoid band have these granules in both the hairs and the upper epidermal cells of the corollas. Flowers with carotenoid granules in the upper epidermal cells vary in appearance from orange

TABLE I. Anthocyanin pigments found by chromatographic analysis in the races and hybrids of the *Mimulus cardinalis* complex

Area of corolla examined	Species or hybrid	Culture number	Origin of cultures or parentage of hybrids	#1 pink R_f=0.45	#2 purple R_f=0.40	#3 lavender R_f=0.36	#4 pink R_f=0.32	#5 blue R_f=0.24	#6 magenta R_f=0.19
Whole corolla:	*M. cardinalis* Dougl.*	5031	Beaver Creek, Siskiyou County, California				x	x	x
		5077	Edinburgh Botanic Garden, Scotland	x		x	x	x	x
		5109	Liége Botanic Garden, Belgium	x‡			x	x	x
		5111	Basel Botanic Garden, Switzerland	x			x	x	x
	M. lewisii Pursh.†	5032	Porcupine Flat, Mariposa Co., Calif.						x
		5051	Fallen Leaf Lake, El Dorado Co., Calif.					x	x
		5875	Storm Mountain, Salt Lake Co., Utah		x	x		x	x
	M. eastwoodiae Rydb.	5848	Arches National Monument, Utah		x	x	x	x	x
	F_1 hybrids	5807	5077 × 5031	x		x	x	x	x
		5810	5031 × 5111	x			x	x	x
		5627	5031 × 5032				x	x	x
		5635	5051 × 5031				x	x	x
		5636	5051 × 5077	x		x	x	x	x
		5812	5032 × 5109	x			x	x	x
		5813	5109 × 5032	x			x	x	x
		5814	5077 × 5032	x		x	x	x	x
		5816	5032 × 5031				x	x	x
		5817	5077 × 5051	x		x	x	x	x
	F_2 hybrids	680	5636 × self	41/43		31/43	40/43	43/43	43/43
Corolla lobes:	*M. cardinalis*	5077	parent	x		x	x		
	M. lewisii	5051	parent					x	x
	F_1 hybrid	5817	5077 × 5051	x			x	x	x
	F_2 hybrid	680	5636 × self	39/40		13/40	39/40	32/40	32/40
Corolla throat:	*M. cardinalis*	5077	parent	x		x	x	x	x
	M. lewisii	5051	parent					x	x
	F_1 hybrid	5817	5077 × 5051			x	x	x	x
	F_2 hybrid	680	5636 × self	11/40		29/40	37/40	40/40	40/40

* All cultures are populaitons of the California race.
† Cultures 5032 and 5051 are populations of the Sierra Nevada race whereas 5875 is a population of the Rocky Mountain
‡ A weak band compared to the #1 bands of 5077 and 5111.

to red, whereas flowers without them vary from lavender to pink. These observations on the phenotypic effect of the carotenoid granules confirm those made by Brozek[1], on several horticultural populations of *M. cardinalis* hybrids. We found that the presence of the granules in the epidermal cells appears to be controlled by a pair of dominant epistatic genes originally present in *M. lewisii*. When one or two of the dominant alleles are present, the carotenoid granules are limited to the corolla hairs and do not appear in the corolla epidermal cells. This simple genetic difference determines whether the color will be in the red series or in the pink series.

The xanthophyll and anthoxanthin pigments also show quantitative differences in intensity. These pigments are being studied further in order to clarify their modes of inheritance and their effects on the phenotype.

Genetics of Anthocyanin Pigment Production

An inspection of the distribution of the anthocyanins as shown in Table I reveals interesting comparisons between the two original parents and the other populations, races, and species of the complex. The original *M. cardinalis* population (culture number 5077) has five of the six anthocyanin bands, whereas the other three *M. cardinalis* populations have only three or four. The 5077 population appears the darkest red of the group. *M. eastwoodiae* Rydb. has the same combination of pigments as 5077, but the pigment bands are weaker which gives it a lighter red phenotype than that of 5077. The original *M. lewisii* population (number 5051) has only two anthocyanin pigments. The other population of the California race (number 5032) has only one pigment. Both of these races are pale lavender pink in contrast to the bright magenta of the race of *M. lewisii* from the Wasatch Mountains. The latter race has four bands including the distinctive purple *#2* band. Each population studied can be distinguished from any of the others by the number and intensity of the anthocyanin pigment it contains.

The first step in the analysis of the modes of inheritance of the anthocyanin pigments was to determine the distribution of the pigments in the available F_1 hybrids, (see Table I). No conclusions were possible concerning either the purple *#2* pigment, since it was not present in any of the parents of the F_1 hybrids, or the magenta *#6* pigment, which was uniformly present in all of the populations and hybrids examined. The F_1's show the #1, 3, 4, and 5 anthocyanin pigments, even though one of their parents lacked one or more of them. Thus, dominant genes appear to control the presence of all of these pigments.

In order to carry the analysis of the modes of inheritance further, it was necessary to study the pigment distribution in the F_2 generation. Only one F_2 population was studied. The parent races, 5077 and 5051, differ with respect to bands #1, 3, and 4; but since both races contain band # 5, it was impossible to carry the analysis of the inheritance of that band any further.

An inspection of the first part of Table I shows that in the investigation of the anthocyanin pigments present in the whole corollas of the F_2 population, the two pink bands, *#1* and *#4*, appeared in 41 out of 43 individuals and in 40 out of 43 individuals respectively; whereas the lavender *#3* band appeared in 31 out of 43 individuals. The F_2 ratios suggest that band *#1* is controlled by two or more pairs of genes, band *#3* by a single pair of genes, and band *#4* by two or more pairs of genes. These conclusions, which indicate that the anthocyanin pigments are controlled by at least five and probably more than seven pairs of genes, contrast with Brozek's[1] explanation which involved only two pairs of genes. However, his interpretation was based on phenotypes alone and not on the individual component pigments.

A careful examination of the chromatograms revealed striking and significant differences in the intensities of the *#1* and *#4* pink anthocyanin bands. For example, the chromatogram of the F_1 (5077 $\times$ 5051) has pink bands that are approximately one-half the intensity of the pink bands in the chromatogram of the 5077 parent (*M. cardinalis*). The F_2 individuals' chromatograms show various intensities of pink pigments. The intensities could be arranged in five categories:

1) equal to that of the *M. cardinalis* parent,
2) intermediate between that of the *M. cardinalis* parent and that of the F_1 hybrid,
3) equal to that of the F_1 hybrid,
4) less than that of the F_1 hybrid, and
5) zero like that of the *M. lewisii* (5051) parent.

These differences suggest that each of the pink anthocyanin pigments is controlled by two pairs of genes that act in a quantitative manner. Additional F_1 and F_2 hybrids are now being grown in order to clarify the modes of inheritance of all of the anthocyanin pigments.

Genetics of Anthocyanin Pigment Distribution

Since the corollas differ in the arrangement and intensity of red and purple dots and streaks, we thought that chromatogramming different areas of the corollas separately might be informative. The corolla lobes, all of which lack these dots and streaks, were chromatogrammed first. The distributions of the pink pigments, *#1* and *4*, were unchanged, whereas the distributions of the *#3*, *5*, and *6*, pigments were markedly altered.

The lavender *#3* band was absent in the corolla lobes of *M. lewisii*, the F_1, and 27 out

of 40 of the F_2 (see Figure 1). These data can be explained by assuming the presence in the *M. lewisii* parent of a pair of dominant genes that prevents the expression of the #3 pigment in the corolla lobes. The interaction of the distribution genes and the pair of genes controlling pigment production (see above) implies that the F_2 will exhibit three phenotypes: pigment present in the corolla throat only, pigment present in the whole corolla and pigment lacking in the whole corolla. The three phenotypes appear in a ratio of 16:13:11, respectively, which is probably only a chance variation, due to the small sample size, of the expected ratio of 9:3:4.

The #5 band was absent in the corolla lobes of *M. cardinalis* and eight out of 40 F_2 hybrids. We interpret these data to indicate the presence in *M. cardinalis* of a pair of recessive distribution genes that prevent the appearance of the pigment in the petal tips. In as much as both parents, the F_1, and the F_2 are homozygous, apparently, for the production of the #5 pigment the distribution genes produce a simple case of recessive monohybrid inheritance. The expected F_2 ratio of three with the pigment present in the whole corolla to one with the pigment present in the corolla throat only was found almost exactly.

The #6 anthocyanin band behaved in the same way as the #5 band. On first thought, the distribution of both bands appears to be under the control of the same pair of recessive distribution genes. Further study showed that different F_2 individuals lacked the #5 and #6 pigments. Apparently, the distribution of each band is under the control of independent pairs of distribution genes.

The corolla throats, which contain the dots and streaks, were chromatogrammed separately also. The distribution of the #3, 4, 5, and 6 anthocyanin pigments was unchanged, whereas the distribution of the #1 pink band was strikingly altered. The #1 band was absent in the corolla throats of *M. lewisii*, the F_1, and 29 out of 40 F_2 hybrids. These data can be explained by assuming the presence in *M. lewisii* of a pair of dominant distribution genes that prevent the expression of the pigment in the corolla throat. The interaction of the distribution genes and the two pairs of quantitative genes that control the production of the #1 anthocyanin pigment implies that the F_2 will exhibit three phenotypes: pigment present in the whole corolla, pigment present in the corolla lobes only, and pigment lacking in the whole corolla. The three phenotypes appear in a ratio of 11:28:1, respectively, which is remarkably close to the expected ratio of 15:45:4.

Thus chromatogramming the different areas of the corollas separately did not elucidate the inheritance of the dots and streaks as had been hoped, but it did reveal interesting data that indicate the presence of two pairs of recessive genes in *M. cardinalis* and two pairs of dominant genes in *M. lewisii* that control the distribution of four different anthocyanin pigments. The interrelationships of the genes controlling the distribution of the anthocyanin pigments and the genes controlling their production are summarized graphically in Figure 1.

Conclusions

Table II summarizes all the preceding data on the various modes of inheritance of the different component flower color pigments: the anthocyanins, anthoxanthins, xanthophylls, and carotenoids. Upon studying that table we can begin to understand the complex genetic situation which produced the large number of phenotypes originally observed. Thus, as a first approximation of the mode of flower color inheritance in the *M. cardinalis* complex, we can report that:

1) at least ten pigments are involved: 1 carotenoid, 1 xanthophyll, 2 anthoxanthins, and 6 anthocyanins;
2) six and probably more of the pigments are controlled by quantitative genes;
3) at least one of the pigments, the #3 anthocyanin, is controlled by a single pair of genes and at least two of the pigments, the #1 and 4 anthocyanins, are controlled by two or more pairs of genes; and
4) the locations of at least five of the pigments, the carotenoid and #1, 3, 5, and 6

TABLE II. Modes of inheritance of the component flower color pigments chromatographically detected in the *M. cardinalis* complex

Component pigments:				Production controlled by:		Distribution controlled by:			
Class of pigment	Band #	Band color	R_f value	No. of genes	Type of gene action	No. of genes	Type of gene action	Area lacking the pigment	
Carotenoids:	1	yellow	1.0	no data	quantitative	1 pair	dominant	corolla	epidermis
Xanthophylls:	1	yellow	1.0	no data	quantitative	none	—	—	—
Anthoxanthins:	1	yellow	1.0	no data	quantitative	none	—	—	—
	2	yellow	0.75	no data	quantitative	none	—	—	—
Anthocyanins:	1	pink	0.45	2 pairs	quantitative	1 pair	dominant	corolla	throat
	2	purple	0.40	no data	no data	no data	—	—	—
	3	lavender	0.36	1 pair	dominant	1 pair	dominant	corolla	lobes
	4	pink	0.32	2 pairs	quantitative	none	—	—	—
	5	blue	0.24	no data	dominant	1 pair	recessive	corolla	lobes
	6	magenta	0.19	no data	no data	1 pair	recessive	corolla	lobes

anthocyanins are under the control of dominant or recessive distribution genes.

Further work is in progress to amplify, clarify, and test these conclusions. However, taken together, all of the data thus far presented give us a preliminary explanation of the beautiful and striking array of flower colors observed in the *Mimulus cardinalis* complex.

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THE INHERITANCE OF LEAF SHAPE IN KENAF

J. Frank Joyner and James B. Pate*

KENAF, *Hibiscus cannabinus* L., passes through a period of juvenile leaf growth, following the cotyledonary stage in which the first 8-12 leaves are unlobed. Leaves above this point become progressively lobed into three, then five and eventually seven lobes. The climax seven lobed leaf becomes stabilized until the onset of flowering, after which there is a progressive reduction in lobes from seven to five and three and sometimes to an unlobed leaf more or less linear in shape. Margins of all leaves except the cotyledons are serrated.

There are two degrees of lobing in kenaf. In some types lobing is deep and a compound leaf structure is approached, while in others the lobing is very shallow and an entire leaf form is maintained. These two leaf forms have been referred to in the literature as palmately lobed or divided and cordate with shallow lobes or entire, respectively[1,2,4]. Since both forms are palmately lobed, and the difference is in degree of lobing, the use of deep *vs.* shallow lobing appears more desirable than any of the above-mentioned terms. This paper concerns the inheritance of degree of lobing using selfed lines found to be breeding true for the lobing character.

Deshpande[3] found a lobed (deep lobed) plant in an entire (shallow lobed) variety of kenaf, and it was assumed to be an outcross with a deep lobed variety. The F_2 generation and F_3 progenies resulting from the outcross segregated in such a manner as to suggest dominance of deep lobing and inheritance on a monofactorial basis. In *Hibiscus rosa-sinensis*, juvenile leaves may be lobed or unlobed, and the presence of lobes has been found to be dominant with one single gene pair involved in the inheritance[5].

KENAF LEAF SHAPE

Figure 2

The deep lobed leaf shape in kenaf exhibits complete dominance. Left to right: BG-52-29, F_1 of BG-52-29 × BG-52-31, and BG-52-31.

Methods

Two deep lobed lines and two shallow lobed lines were crossed reciprocally in all possible combinations. These four lines were second generation selfs out of the Salvadorian variety of kenaf. F_1's were grown and observed, and one of these was selected for selfing and backcrossing to study segregation in the F_2 and backcross populations. Selfs were made in the F_2 and backcross populations to obtain seed of F_3 and backcross progenies.

Results

The leaf shape classifications of parents and F_1's are presented in Table I. The deep lobed shape was completely dominant throughout this series of crosses. Reciprocal crossing did not alter this pattern of dominance. The complete dominance of the deep lobed shape in the climax phase is shown in Figure 2.

(*Continued on page* 204)

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BRACHYDACTYLIA IN MICE

LAWRENCE B. HARDY AND LEONELL C. STRONG*

A SERIES of papers reporting the appearance and partial genetic analysis of many color and morphological characteristics in mice whose ancestry has been injected with methylcholanthrene for many generations has already been published.[6-14]

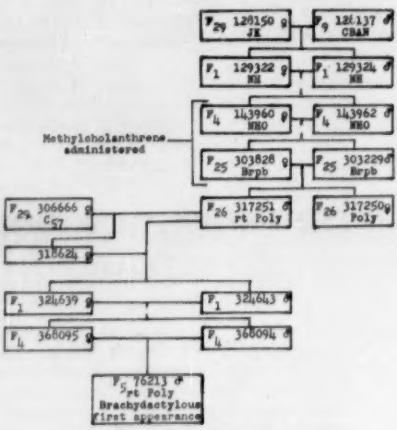
The present paper deals with a new morphological character of brachydactylia which appeared in Strong's polydactylous strain of mice[2] of the present methylcholanthrene-treated descent. Due to the fact that all mice tested showing brachydactylia have been proven to be sterile, it is very difficult to make a complete genetic analysis of the character.

There are, therefore, two purposes in presenting the present paper: (1) to record the appearance of 16 cases of brachydactylia in a restricted single descent from an ancestral methylcholanthrene-treated ancestry, and (2) to describe the new character with a comparison of a similar, although not identical condition, known as brachypodia, which has recently been described in mice.[3]

Materials and Methods

The polydactylous strain in which the brachydactylous mice arose was the result of a cross of a polydactylous strain male (F_{26} 317251) to a female C_{57} (F_{29} 306666). The C_{57} was originally from Bagg and had been inbred by Strong for 29 generations at the time of this outcross. The "poly" strain is a subline of the NHO mice which were in turn separated from the NH strain. The origin of the NH mice has been previously noted.[1,4,5]

Briefly stated, the origin of this polydactylous strain is as follows. Mice of the CBA and N strains were crossed.



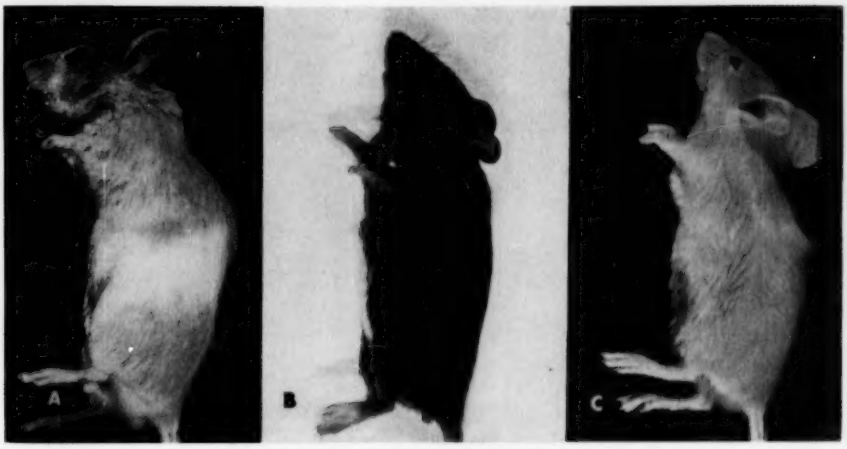
PEDIGREE OF THE BRACHYDACTYLOUS SUBLINE

Figure 3

The condensed pedigree is somewhat schematic. The first brachydactylous animal occurred in the F_5 generation of a cross between a female (F_{29} 306666) of the C_{57} strain and a male (F_{26} 317251) of the polydactylous stock. It is to be noted that mice of this descent were injected with methylcholanthrene at sixty days of life for a period of approximately 12 years (from 7/29/1939 to 9/1/1951). Two polydactylous mice appeared about seven months after the last lineal descent female had been injected with this carcinogen. Following the appearance of polydactylia no further injections of methylcholanthrene were made.

After nine generations of sib-matings, a male mouse of the F_9 generation (128137 male) was outcrossed to a female mouse of the JK (F_{29} 128150 female) inbred stock. In the F_4 following this outcross the mice were divided into four groups. Of these four groups, three were maintained as controls by brother-sister matings. The fourth group was injected subcutaneously at 60 days of age with one mg. of methylcholanthrene dissolved

*Biological Station, Roswell Park Memorial Institute, Springville, New York. This experiment has been made possible, in part, by a grant administered through Health Research, Inc. from the Committee on Growth of the National Research Council, acting for The American Cancer Society—grant terminated October 27, 1955.



COMPARATIVE FOOT LENGTH

Figure 4

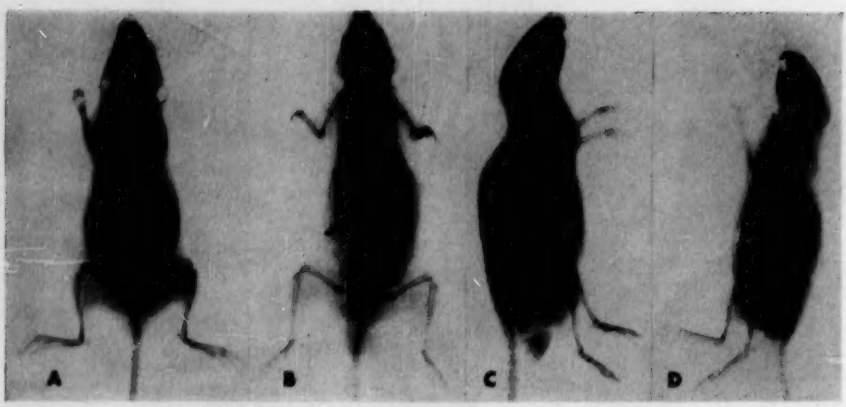
Illustrated is the difference in foot length in mice of the (*A*) pBr strain for comparison (*B*) brachydactylous in one of its ancestral strains and (*C*) a mouse of an entirely unrelated strain (the I-strain).

in one cc. of sesame oil and continued by sib-matings as breeders. This later descent constitutes the NHO subline (descendents of F_4 143960 male $\times$ F_4 143962 female). The pBr and its several sublines, Prunt, etc., are of this same descent. The NHO were then separated during 1943 into four further sublines, the pBr, Br, Brpb, and pBrpb. The F_{18} 259103 female (Brpb subline) was continued and her descendent seven generations later the F_{25} 308328 female was the mother from which the F_{26} 317251 male poly arose (see Figure 3). This male was then crossed to several C_{57} females among which was F_{29} 306666. In the F_5 following this last outcross mouse F_5 76213 male was obtained and this was seen to be a brachydactylous mouse. For a complete history see Figure 3. Fifteen other cases of brachydactylia have since occurred in collateral lines to the above one.

The skeletal morphology of the mice used in this study was determined on both killed and living specimens.*

The killed material was fixed in 95 percent alcohol and stained with alizarine red (Dawson's Method—modified by Hardy) in the following manner. The mice were killed by etherization, skinned and eviscerated, and then fixed in 95 percent alcohol for 24 to 48 hours (depending on the size of the mouse). From the alcohol the mice were placed in acetone for purposes of de-fatting for 72 to 80 hours. The mice were then cleared in a 1.0 percent solution of KOH until all the bones could be clearly seen through the muscle tissue—about 7 to 10 days. The mice were then placed in 1:10,000 alizarine stain (10,000 H_2O) for 3 to 12 hours or until stained the desired degree of red. Destaining is difficult so it is necessary to keep close observation during the staining process. If the tissue is properly cleared (left in KOH 24 hours longer than is thought necessary) the stain will infiltrate the bone only and leave the other tissue clear. The specimens were then transferred to a glycerine solution in the fol-

*The X-ray photographs and the direct photographs of the living material were prepared by Mr. Charles Herger, technician, diagnostic X-ray department and Mr. William Payne, department of medical photography, both of the Roswell Park Memorial Institute, Buffalo.



NORMAL AND BRACHYDACTYLOUS MICE

Figure 5

X-ray comparison of the normal feet and the brachydactylous feet of mice described in the text. *A* and *D* are normal; *B* and *C* brachydactylous.

lowing proportions: 20 ml. glycerine, 79 ml. H_2O and 1 ml. of 1 percent KOH. The specimens were kept in this solution 24 to 36 hours or until the specimens no longer bled stain. From this point on the mice were carried up through glycerine in 40 percent, 60 percent, 80 percent, 90 percent aqueous solutions and then finally stored in 100 percent glycerine.

The measurements of the mice made possible by either the alizarine stain method or the X-ray photographs were made of the bones only and were taken by vernier calipers with the aid of a dissecting microscope.

All the mice used in this study were produced in this laboratory. Mice were kept in uncrowded wooden boxes and were given a diet of Nurishmix supplemented by mixed grain. Once a week they were also given a piece of bread soaked in milk to which cod liver oil had been added. Water was given *ad libitum*.

Results

Sixteen cases of brachydactylia have appeared between the F_4 and F_9 generations following a cross (Figure 3) between polydactylous male mouse 317251 to female mice of the C_{57} strain. These data are recorded in Table I.

Five male brachydactylous mice were tested for potency against normal polydactylous females and brachydactylous females. They were allowed sufficient time for breeding. Therefore, it is concluded that the male brachydactylous mice were sterile.

Description

The morphological mutations and changes in the house mouse already described in the literature have been enormous. After a rather thorough review of the literature it became obvious that there was only meager knowledge of the condition, brachydactylia, in the mouse. Indeed it was only possible to find one reference, and that was to a slightly different condition called brachypodism described by Landauer[8]. The condition which Landauer describes is inherited in the mouse as an autosomal, recessive mutant. As far as the authors know this is the only reference in the literature which describes a reduction in the length of the feet of certain mice. We will describe,

TABLE I. Occurrence of 16 brachydactylous mice between the F_4 and F_9 generation

Generation	Mouse No.	Age of mother*	Normal: Brachy	No. brachy, litter rank and age of mother*
F_9	66468	61	47:1	(1) 4 at 133
F_7	69648	289	57:1	(1) 4 at 128
F_6	69659	225	59:2	(1) 3 at 117
				(1) 6 at 193
F_6	76719	294	67:1	(1) 1 at 54
F_6	65860	174	48:2	(2) 5 at 181
F_4	68095	264	39:4	(1) 1 at 64
				(3) 3 at 284
F_5	68130++	213	48:2	(1) 1 at 96
				(1) 3 at 170
F_5	68131	213	48:1	(1) 5 at 172
F_5	52720++	166	62:2	(1) 9 at 266
				(1) 11 at 343

*Age of mother when litter containing a brachydactylous mouse was born.

in this paper, a condition which is somewhat like that of Landauer, however, the condition differs markedly in many respects.

The gross appearance, except for the feet, of these brachydactylous mice, is in all respects, like that of all normal mice of other strains. It is only when the animal is examined carefully that the reduction in foot size is noticed. The gross appearance of the legs are otherwise like those of any other mouse (normal) in the parent strain. The reduction when present is always noted on all four feet. The hind feet may, however, be a little more affected with brachydactylia than the fore feet. It will be seen in Figure 4 that the brachydactylous foot length differs significantly from that of mice of the ancestral strains (JK, pBr). In Table II is listed the actual data of the size of the feet in the polydactylous, JK, pBr, A and I strains of mice. The JK and pBr are ancestral strains of the polydactylous strain from which arose the brachydactylous line. (See Figure 3.)

In the brachydactylia strain there is a definite shortening of both the fore and hind feet. The average length for the fore foot is 0.51 mm. in the brachy and 0.76 mm. in the normal poly, (see Table II). In the hind feet we find a very marked reduction in foot length, the brachy is 1.20 mm. as opposed to the normal mice which measure 1.82 mm. There is a slight reduction in the size of the carpels and metacarpels, and tarsals and metatarsals but this reduction does not appear to be significant. It would seem that the reduction in foot length is mainly in the digits themselves. There may be a slight tendency toward an occasional extra metacarpel or metatarsal. However, in as much as these brachys arose out of the polydactylous strain this would not be unexpected. We do not find that these small bones of the feet are abnormal in shape and we do not find any tendency toward bifurcation. The digital bones are normal in number although each is greatly reduced in size. The digital bones do not have any tendency to bifurcation at their areas of articulation. Figure 5 is a representative group of X-ray photographs showing the normal and the brachydactylia condition.

TABLE II. The mean average, measured in mm. of the fore and hind feet of brachydactylous and normal mice in the strains listed

	Brachy	Poly	JK	pBr	A	I
Forefeet	0.51	0.76	0.85	0.90	0.80	0.80
Hindfeet	1.20	1.85	1.97	2.10	2.15	1.98

TABLE III. Measurements in mm. taken from X-ray photographs of the bones or of the bones stained by the alizarine stain (modified by Hardy)

	Brachy	Poly	JK	pBr	A	I
Humerus	0.95	1.03	1.15	1.12	1.17	1.15
Ulna	1.10	1.15	1.30	1.20	1.25	1.15
Femur	1.00	1.16	1.35	1.10	1.00	1.10
Tibia	1.40	1.48	1.70	1.75	1.75	1.65

Table III shows that there is no significant reduction in the length of the long bones in fore legs or hind legs. The remainder of the skeleton does not appear to be affected in any way. The internal anatomy of brachydactylous mice does not vary from the normal mouse.

General Discussion

The appearance of brachydactylia on all four feet of all 16 cases is evidence that the condition occurs very early in embryonic development. That all individuals tested proved to be sterile is further evidence that some other internal defect is also present, the nature of which being still unknown. All brachydactylia mice also showed polydactylia but this finding is probably co-incidental since the "short toes" occurred in mice of a strain showing approximately 75 percent penetrance of "extra toes."

The ratio of affected mice to normal obtained (by crossing two normal mice together produced 475 normal : 16 brachydactylia or *29.69*:1) is evidence of a complex method of inheritance but this mechanism cannot be further analyzed due to complete sterility of all brachydactylous individuals.

Summary

Brachydactylia is similar, but not identical, with brachypodia reported by Landauer. The effect upon the length of the various components of the foot and leg and the mode of inheritance are apparently different in the two characters. Brachypodia is inherited as a simple recessive whereas brachydactylia is apparently a complex recessive.

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The Inheritance of Leaf Shape in Kenaf

(*Continued from page* 199)

The F_1 of BG-52-29 and BG-52-31 was selected to study segregation in the F_2 and backcross populations and in F_2 and backcross progenies. The segregation results obtained are presented in Tables II and III. In the backcross to the shallow lobed parent a 1:1 ratio was obtained. The F_2 segregated into a ratio of three deep lobed to one shallow lobed. Backcross and F_2 progenies segregated as expected assuming dominance of deep lobing and inheritance on a monofactorial basis.

TABLE I. Classification of kenaf parental lines and F_1's for leaf shape

Line or F_1	Deep Lobed	Shallow Lobed
BG-51-1	0	20
BG-52-31	0	26
BG-51-3	21	0
BG-52-29	28	0
BG-51-1 × BG-52-31	0	15
BG-52-31 × BG-51-1	0	7
BG-51-1 × BG-51-3	20	0
BG-51-3 × BG-51-1	20	0
BG-51-1 × BG-52-29	20	0
BG-52-29 × BG-51-1	10	0
BG-52-31 × BG-51-3	12	0
BG-51-3 × BG-52-31	14	0
BG-52-31 × BG-52-29	30	0
BG-52-29 × BG-52-31	12	0
BG-51-3 × BG-52-29	24	0
BG-52-29 × BG-51-3	11	0

TABLE II. Classification of kenaf backcross and F_2 populations for leaf shape

Population	Number of Plants: Deep Lobed	Shallow Lobed	Assumed Ratio	χ^2	P
(BG-52-31 × BG-52-29) × BG-52-29	185	0			
(BG-52-31 × BG-52-29) × BG-52-31	74	56	1:1	2.49	.18
BG-52-31 × BG-52-29 F_2	298	87	3:1	.83	.42

TABLE III. Classification of kenaf backcross and F_2 progenies for leaf shape

Population	Number of Progenies: Deep Lobed	Segregating	Shallow Lobed	Assumed Ratio	χ^2	P
(BG-52-31 × BG-52-29) × BG-52-29	13	17	0	1:1	.53	.48
(BG-52-31 × BG-52-29) × BG-52-31	0	19	8			
BG-52-31 × BG-52-29 F_2	19	33	18	1:2:1	.26	.61

Summary

The inheritance of leaf shape in kenaf was studied. Deep lobing was found to be completely dominant to shallow lobing. Segregation in F_2 and backcross populations and in F_2 and backcross progenies showed inheritance to be on a monofactorial basis.

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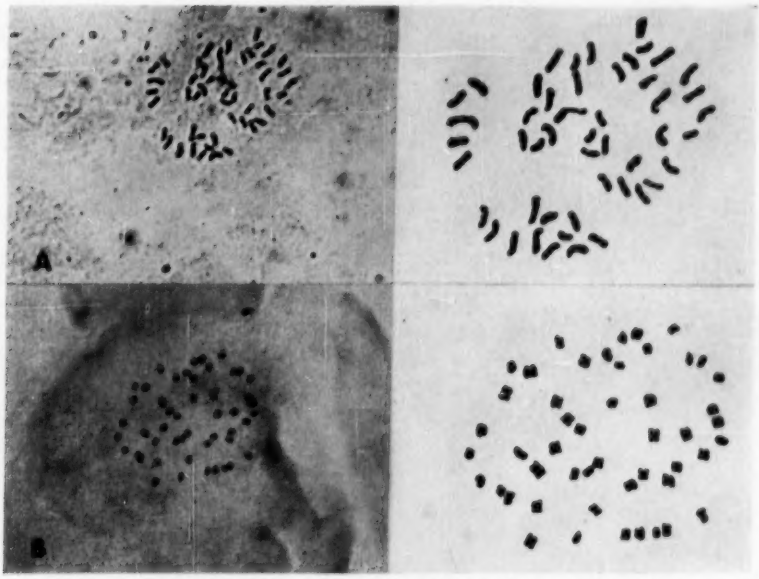
MITOTIC INSTABILITY IN BLACKBERRY SEEDLINGS

From Progenies of Boysen and of Young

DONALD M. BRITTON AND J. W. HULL*



MITOTIC CHROMOSOMES OF BOYSEN AND OF YOUNG

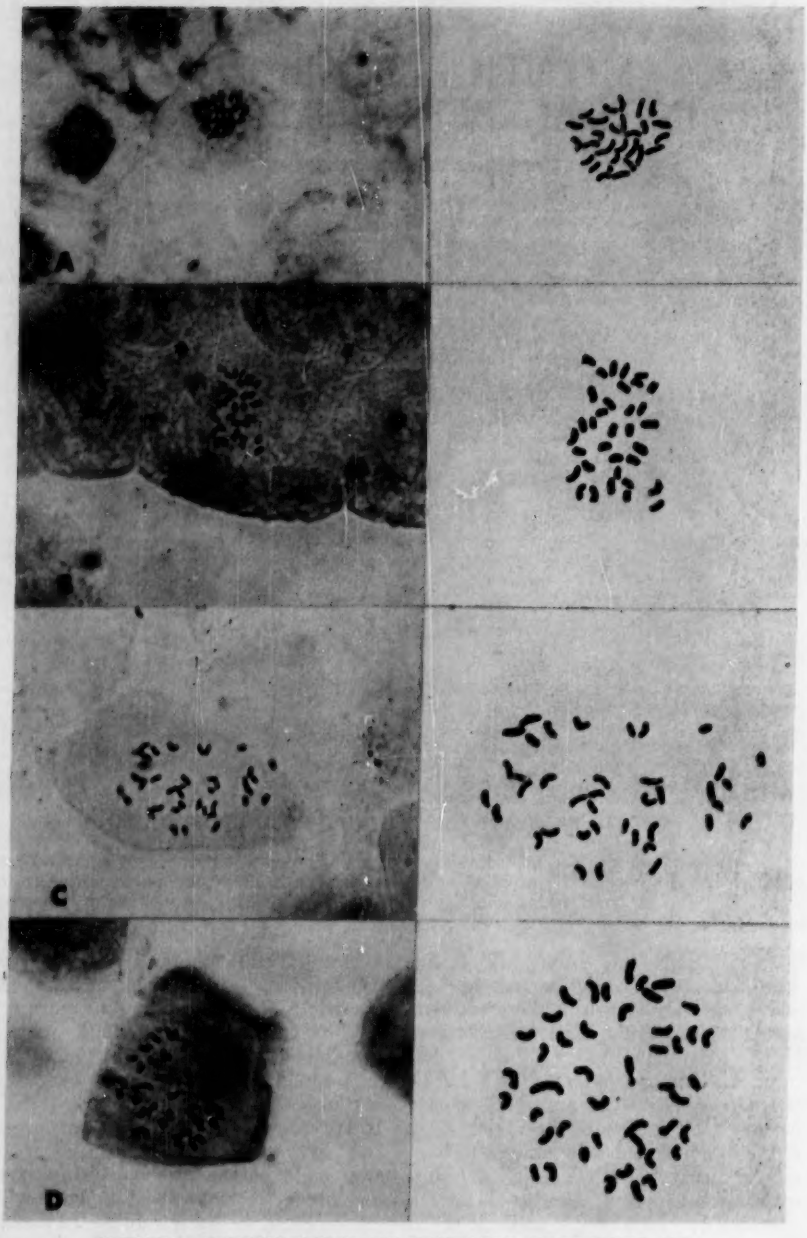
Figure 6

A—Photomicrograph and photodrawing of 49 somatic chromosomes of Boysen. *B*—Photomicrograph and photodrawing of 49 somatic chromosomes of Young from root-tips pretreated with P.D.B. for one hour. (Photomicrographs approximately 1130×, photodrawings[11] approximately 2000×).

BOYSEN and Young are trailing blackberry varieties that are extensively grown in the more temperate regions of the United States from North Carolina to California and Oregon. Young originated as a hybrid between Phenomenal and Mayes dewberry[2, 3]. The origin of the variety Boysen is in doubt, but it is thought to be of similar nature[3]. Both Young[4] and Boysen[8] are reported to be hexaploid ($2n=42$). However, collections of Thornless Boysen, Boysen, and Young from the sources indicated in Table I were studied cytologically and clear mitotic figures were obtained from each showing 49 chromosomes (Figure 6 *A* and *B*), indicating that these varieties are heptaploid.

Boysen and Young have been used extensively in a breeding program at this Station; however, only two crosses will be considered here. In these crosses the male parent was colchicine-treated Eldorado, an eastern blackberry which is reported to be tetraploid ($2n=28$)[2]. In the course of other studies hundreds of root-tips of Eldorado were examined

*Contribution of the University of Maryland Agricultural Experiment Station, Department of Horticulture. Scientific Article No. A543.



MITOTIC CHROMOSOMES OF UNSTABLE PROGENIES OF BOYSEN AND OF YOUNG

Figure 7

A-E—Photomicrographs and photodrawings of somatic chromosomes of Plant No. 1, Table

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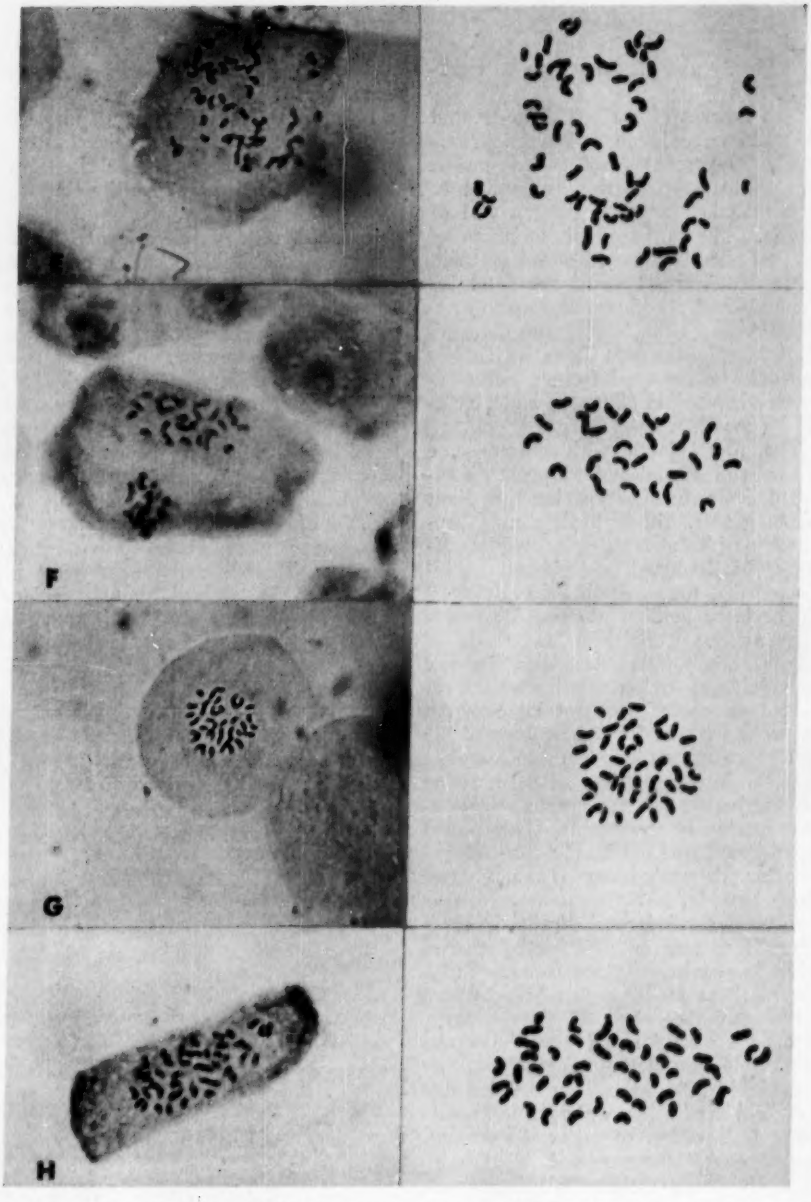

III (Boysen × Eldorado). *A*—28 chromosomes from serial root-tip section. *B*—Pretreated 30 minutes with P.D.B., 34 chromosomes. *C*—39 chromosomes. *D*—45 chromosomes. *E*—60 chromosomes. *F* and *G*—Photomicrographs and photodrawings of somatic chromosomes of Plant No. 4, Table III (Young × Eldorado). *F*—27 chromosomes. *G*—40 chromosomes. *H*—Photomicrograph and photodrawing of somatic chromosomes of plant grown from leaf-bud cutting of Plant No. 1, Table III, with 48 chromosomes. (Photomicrographs approximately 1130×, photodrawings approximately 2000×).

cytologically and all proved to be tetraploid.

Two flowers of the colchicine-treated Eldorado were found to have approximately 30 percent giant pollen grains. The pollen from these was applied to emasculated flowers of Boysen and of Young. The chromosome numbers of the two resulting progenies are given in Table II. Fifty-eight of these 62 individuals had chromosome numbers between 35 and 42 inclusive, with the peak of distribution around $2n=38$. This is as would be expected from a cross between a heptaploid ($2n=49$) and a tetraploid ($2n=28$).

The four plants with chromosome numbers of 49, 50 and 51 probably resulted from fertilization by the giant pollen grains, although they may represent accidental selfs or apomictic offspring of Young. Their phenotypes indicated that they were hybrids.

The large number of aneuploid seedlings arising from these crosses is of interest when contrasted with the few aneuploids of *Rubus* that have been reported as occurring naturally or from controlled crosses[6,7,18]. The lack of reported aneuploids led Kerr[10] to postulate that the low percentage of germination of blackberries is due in part to the non-germination of aneuploids. This postulate is not borne out by the chromosome number determinations obtained from these hybrids.

Einset[7] studied cytologically 59 seedlings from two open-pollinated species of *Rubus* having 49 chromosomes. Fifty-six of these seedlings had 49 chromosomes and three had 63 chromosomes. From these results, and from the results of examination of the progenies of other polyploid blackberries, he concluded that "Hybrid seedlings resulted infrequently after fertilization of reduced or unreduced eggs." Boysen and Young apparently do not behave in this manner.

Mitotic Instability in the Progenies

Six plants not included in Table II (three of Boysen × Eldorado and three of Young × Eldorado), were studied in greater detail because different chromosome numbers were found in different roots or even in the same root.

The number of mitotic figures for each of the different chromosome numbers obtained is shown in Table III. Only those mitotic figures where the chromosome number could be ascertained within an error of plus or minus one chromosome are presented. Other chromosome numbers have been found in these plants but the determinations were not within this arbitrary limit. Because of the difficulty of the material, there are presented fewer determinations of chromosome numbers over 50 than actually were seen. Such determinations in this material can usually only be made within an error of plus or minus two chromosomes.

Most of the determinations reported in this paper were made from root-tip squashes. Root-tips were fixed in acetic-alcohol (1:3), stained by the Feulgen schedule[1], and squashed in iron aceto-carmine. Two series of serial sections of 10 root-tips each also were prepared. The root-tips were fixed in CRAF fixative and were stained by the Feulgen schedule followed by La Cour's crystal violet schedule[1]. This method produced brilliantly stained chromosomes with a

TABLE I. Sources of Boysen and of Young

Number	Variety	Source
1	Thornless Boysen	Department of Horticulture, Oklahoma A. & M., Stillwater, Okla.
2	Boysen	Stark Brothers Nursery, Louisiana, Missouri
3	Boysen	Carlton Nurseries, Forest Grove, Oregon
4	Boysen	George M. Darrow, Glenn Dale, Maryland
5	Young	Carlton Nurseries, Forest Grove, Oregon

TABLE II. Chromosome numbers of progenies of Boysen and of Young

Chromosome Number	Number of Progeny	
	Boysen × Eldorado	Young × Eldorado
35	6	2
36	6	1
37	5	2
38	9	4
39	8	3
40	3	4
41	1	1
42	1	2
49	0	2
50	0	1
51	0	1
Totals	39	23

clear background, but "end-to-end" associations of chromosomes were the rule (Figure 7 *A*). Few mitotic figures from sectioned material are included in Table III because few determinations were possible within the limits of plus or minus one chromosome. The chromosome number determinations obtained from the sectioned material were already represented among the determinations made with the squash technique. Figure 7 *A-E* illustrates some mitotic plates from plant No. 1. Figure 7 *F* and *G* are plates from plant No. 4.

These six plants were all vigorous and did not represent extreme phenotypes of the progeny. The three plants with Young as the female parent were not distinguishable from those with Boysen as the female parent.

Vegetative Propagation of Plant No. 1

Twenty-four leaf-bud cuttings from plant No. 1 (Table III) were rooted in coarse sand under a constant mist. Chromosome number determinations for each of six of the resulting plants were made from root-tip squashes. Clear mitotic plates were obtained from each plant that showed somatic chromosome numbers of 35, 40, 41, 45, 48, and 60, respectively. Although these chromosome numbers were characteristic of each respective plant at this time, the finding of some other chromosome numbers indicated that mitotic instability was present in their roots.

Discussion

In Table III, the frequency of occurrence of any chromosome number has no significance other than to indicate the number of times that chromosome number was determined within the limits of plus or minus one. Some slides or areas of a slide made by the squash technique are inevitably better than others. Consequently, more determinations come from some slides or areas of a slide, than others. Many chromosome numbers could be found in some root-tips, whereas in others only one number could be found. For example, examinations of the first four root-tips of plant No. 4 (Table III) consistently showed mitotic plates of great clarity with 40 chromosomes (Figure 7 *C*). However, the fifth root-tip examined had many different chromosome numbers with some as low as 27 (Figure 7 *F*).

The predominating chromosome number found in each of five of the plants grown from leaf-bud cuttings of plant No. 1 (Table III), was also observed in the root-tips of the mother plant. One, however, had a characteristic chromosome number of 48 (Figure 7 *H*) which was not found in the examination of any of the 52 root-tips of the mother plant. This may have been because of an insufficient number of determinations, or because this chromosome number was present in the top of the mother plant but not in the roots, or it may have arisen later through mitotic instability.

The chromosome numbers present in plant

TABLE III. Chromosome numbers of mitotically unstable progenies of Boysen and of Young*

Boysen × Eldorado						Young × Eldorado					
Plant No. 1 (*52 root-tips*)		Plant No. 2 (*20 root-tips*)		Plant No. 3 (*40 root-tips*)		Plant No. 4 (*22 root-tips*)		Plant No. 5 (*7 root-tips*)		Plant No. 6 (*6 root-tips*)	
Chroms. No.	No. of cells	Chroms. No.	No. of cells	Chroms. No.	No. of cells	Chroms. No.	No. of cells	Chroms. No.	No. of cells	Chroms. No.	No. of cells
27	2	25	3	13	5	27	8	18	1	37	1
28	1	27	6	14	9	29	4	23	1	40	2
29	4	28	7	15	3	34	2	25	1	49	2
30	19	29	4	18	1	38	1	35	1	58	1
31	21	30	3	20	8	39	4	36	9	71	2
32	2	31	4	21	15	40	32	39	1		
33	8	32	2	27	1	41	1	40	2		
34	10	33	1	28	31	43	1	41	2		
35	6	42	4	29	13			42	1		
36	6			30	1			43	1		
40	5			41	1						
41	1										
43	2										
45	2										
46	2										
47	1										
56	4										
58	1										
60	1										

*Only those chromosome number determinations within an error of one.

No. 3 (Table III) are of special interest because some are below the gametic number of Boysen, the female parent, and some are even less than the gametic number of Eldorado, the male parent. Their presence, considered with the range of chromosome numbers found, can not be explained by polysomaty alone, although this may be one factor involved. Rather, they strongly suggest some mechanism for the diminution of chromosome number in these plants.

There are a number of reports in the literature of varying chromosome numbers in a single plant. Vaarama[17] found a range of chromosome numbers from four to 32 in root-tips from seedlings of colchicine-treated *Ribes nigrum*. Snoad[16] reports chromosome numbers from 23 to 83 in root-tips of *Hymenocallis calathinum*. Less marked variations in chromosome number have been reported by Duncan[5], Ono [12], and Kato[9]. The meiotic studies of Sachs[13] on amphiploids of *Triticum*, *Aegilops*, and *Agropyron* showed the presence of chromosome mosaics in the anthers although none were found in the root-tips. Snoad[15] reported a sub-diploid variation of chromosome number in the pollen mother cells of *Helianthemum*. Recently, it has been suggested by Sharma and Sharma[14] that the chromosome number may not be fixed in many species and varieties of plants.

The present study on *Rubus* presents another example of mitotic instability and shows further that asexual propagation of a single unstable individual can result in plants with different characteristic chromosome numbers. The mechanisms causing the variation in chromosome number are being studied in these plants and in other *Rubi* and will be described in a forthcoming publication.

Summary

Cytological studies of Boysen and of Young blackberry root-tips show that their chromosome number is 49, the heptaploid chromosome number for *Rubus*. These two varieties were crossed with the variety Eldorado ($2n=28$), and chromosome number determinations of the progenies indicated that both Boysen and Young breed as heptaploids.

Three plants in each of the two progenies were found to be mitotically unstable, although they were vigorous and were not distinguishable phenotypically from the other plants.

Clonal propagation of one of the progeny with mitotic instability resulted in plants, each of which differed from the other in chromosome number. Mitotic instability was still present but each of the six plants had one predominant chromosome number.

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SELECTION FOR THYROID WEIGHT IN NEW HAMPSHIRE CHICKENS

WILLIAM E. SHAKLEE AND C. W. KNOX*

SHAKLEE and Shaffner[4] have shown that weight response of the thyroid gland of New Hampshire chickens to thiouracil is easily changed by selection. However, according to Shaklee and Shaffner[5] the activity of the thyroid gland as indicated by egg production, fertility, hatchability, incubation period, growth rate, mature body weight, rate of feathering and mortality apparently did not differ in the high and low lines after three generations of selection. Therefore, it was thought that a different basis of selection should be used in order to obtain information on the inheritance of thyroid weight and possible thyroid activity.

Materials and Methods

A selection experiment with New Hampshire chickens was initiated at the Agricultural Research Center with the object of studying the hereditary aspects of thyroid weight. Several methods of estimating thyroid gland activity including thyroid weights are available, but no one method has been demonstrated to be unquestionably superior to the others. In order to conserve time and funds, it was decided to use the thyroid weight of the four-week-old chicks, the assumption being that greater weight indicates greater activity, as a basis for selection of the parental stock. The experiments described by Riddle[3] with pigeons and doves and by Shaklee and Shaffner[4] with chickens constitute the only selection experiments on thyroid weight known to the authors.

The chicks used in these experiments were obtained from the New Hampshire flock maintained at Beltsville. Pedigreed chicks were kept in batteries and fed a standard growing ration composed of: 51.9 percent corn; 10.0 percent flour mids; 3.0 percent alfalfa; 27.5 percent soybeans; 4.0 percent fish meal; 1.5 percent bonemeal; 1.0 percent limestone; 0.5 percent manganized and iodized salt; 0.1 percent A and D oil and 0.5 percent riboflavin. At four weeks of age, they were weighed, sacrificed and thoroughly cooled in a room kept at 32-33°F. The thyroid glands were removed and weighed immediately on a torsion balance type scale. The family thyroid weight was computed as the arithmetic average of each set of full sibs.

Considerable variation in thyroid weight among families was observed. Additional chicks were hatched during the normal hatching season from the families with the greatest average thyroid weights to become parents of a high line. Similarly, a low line was established from families with small average thyroid weights. This report presents observations on the unselected first generation population, full sibs of birds selected to be parents of high and low

TABLE I. Body weights and thyroid weights of unselected four-week-old New Hampshire chicks

Sex	No. of Chicks	Body Wt. (gm)	Thyroid Wt. (mg)	Thyroid Wt. (mg) ÷ Body Wt. (100 gm)
Males	218	367	23.2	6.32
Females	211	338	24.4	7.22
Total or weighted mean	429	353	23.8	6.74

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lines, and unselected offspring of the selected parents.

Results and Discussion

Some characteristics of the unselected population are presented in Table I. The mean body weight was 353 gm. and the thyroid weight was 23.8 mg. The mg. of thyroid weight per 100 gm. of body weight averaged 6.74.

The females had larger thyroid glands, 24.4 mg., than the males, 23.2 mg., and when the ratio of thyroid weight (mg.) to body weight (100 gm.) was considered, the ratio for females was greater than for males, being 7.22 and 6.32, respectively. The difference between sexes was highly significant. The difference in thyroid weight among hatches was highly significant, as shown in Table II, indicating that environmental factors may easily affect thyroid weight. In spite of this, the heritability of thyroid weight was high. The heritability estimate was derived from the analysis of variance as shown in Table II in accordance with the method outlined by Lerner[1] on page 121. The sire and dam effects were both highly significant and the combined heritability estimate was 92 percent. This estimate of heritability is not much higher than is indicated by the results of the first generation of selection described in succeeding paragraphs.

The frequency distribution was significantly skewed in the positive direction ($t = 3.3$), the mode being 21 mg. The range of thyroid weights was 7.7 to 44.7. A highly significant regression of thyroid weight on body weight was found. This was calculated as a linear regression coefficient and was found to be 9.85 mg. per 100 gm. of body weight. The correlation between thyroid weight and body weight was + 0.593 and was highly significant.

The results of one generation of selection are presented in Table III. It will be noted that selection of parental stock for thyroid weight was quite effective. Lush[2] pointed out that a rough estimate of heritability may be obtained by calculating the amount of progress made in each line as a percentage of the amount the selected parents exceeded the average of their generation. If this is done with these data, the figure 77 percent is obtained. This is almost as high as the 92 percent estimated from the analysis of variance discussed previously and again serves to verify the high estimate of heritability.

TABLE II. Analysis of variance of thyroid weights of four-week-old New Hampshire chicks

Source of variation	Degrees of freedom	Mean square
Among hatches	2	472.28*
Between sexes	1	171.79*
Among sires	4	1,026.92*
Among dams within sires	69	75.63*
Individuals	352	23.44

* Significant at the 1% level of probability.

Summary

A selection experiment on thyroid gland weight in four-week-old New Hampshire chickens was initiated at the Agricultural Research Center.

The correlation between thyroid weight and body weight was +0.59 and the linear regression coefficient was 9.85 mg. of thyroid weight per 100 gm. of body weight. The frequency distribution was skewed in the positive direction. The thyroid gland of the female was heavier than that of the male.

The heritability of thyroid weight was estimated at 92 percent by use of full sib and half sib correlations. The high estimate of heritability was supported by results of the first generation of selection, in which 77 percent of the difference between the average thyroid weight of full sibs of the selected parents and of their contemporaries was recovered in the offspring.

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TABLE III. Body and thyroid weights at four weeks of age of full sibs and the unselected offspring of the selected parents

Relationship to Selected Parents	Line	No. of Chicks	Body Wt. (gm)	Thyroid Wt. (mg)	Thyroid Wt. (mg) ÷ Body Wt. (100 gm)
Full Sibs	High	—	184	12.8*	8.52
	Low	—	364	18.2*	4.98
Unselected Offspring	High	444	314	30.3	9.74
	Low	329	282	19.1	7.19

*Unselected mean for parental generation was 23.8 mg: (Table I).

RESULTS FROM CROSSING RED DANE AND RED POLL CATTLE

E. W. McComas, A. C. Cook, and W. M. Dawson*

IN connection with dual-purpose cattle research at the Chinsegut Hill Sanctuary at Brooksville, Florida in 1937, the United States Department of Agriculture began breeding a part of its purebred Red Poll cow herd to two unrelated Red Dane bulls. Later, female progeny of the cross were back-crossed to Red Poll bulls. The calves, regardless of their breeding, received the same care and similar feed so that a comparison could be made of the purebred Red Poll and the crossbred Red Dane × Red Poll calves. Calves of all three groups (purebred Red Poll; ½ Red Dane + ½ Red Poll; ¾ Red Poll + ¼ Red Dane) nursed their dams for the first three days and were then hand fed milk, grain, and hay to a weight of 450 pounds. There were 55 first-cross calves and 65 back-cross calves born in the herd. Figure 8 shows: (*A*) one of the Red Dane bulls; (*B*) one of the Red Poll cows; (*C* and *D*) two of the first-cross calves.

The F_1 crossbred Red Dane × Red Poll heifers averaged 76.2 pounds liveweight at birth or 9.6 pounds a head more than the Red Poll heifers. Moreover, the F_1 crossbred heifers gained an average of 1.50 pounds a head daily from birth to weaning at 450 pounds, liveweight, or 0.14 pounds more a head daily than the Red Poll heifer calves in the same period. In the period from 450 to 650 pounds, the crossbred heifers averaged 1.52 pounds gain a head daily or 0.24 pound more than the Red Poll heifers. The F_1 Red Dane × Red Poll heifers made cheaper gains than the Red Poll heifers from 450 to 650 pounds, for they consumed an average of 575.40 pounds of total digestible nutrients for each 100 pounds gain in liveweight whereas the Red Poll heifers required 643.24 pounds of T. D. N.

In respect to milk production there were 15 head of first-cross ½ Red Dane + ½ Red Poll heifers at Brooksville that completed a 305-day production record for their first lactation with an average of 6073 pounds of milk and 257 pounds of butterfat with an average test of 4.24 percent. One of the Red Dane bulls used in this test sired 13 of these first-cross heifers. The dam of this bull had a production record of 5171 pounds of milk and 176 pounds of butterfat in 313 days as a junior 2-year-old in Denmark. During the first lactation in each instance, five crossbred Red Dane × Red Poll heifers in the 305-day test produced an average of 6242.4 pounds of milk and 262.6 pounds of butterfat which represented 994.3 more pounds of milk and 39.6 more pounds of butterfat than the average produced by their Red Poll dams during their first lactation. In milk production the crossbred Red Dane × Red Poll cows gave significantly more milk than their Red Poll dams. They also gave more butterfat than their dams but the number of animals was so small that the difference could not be shown to be significant. The five back-cross heifers (¾ Red Poll + ¼ Red Dane breed-

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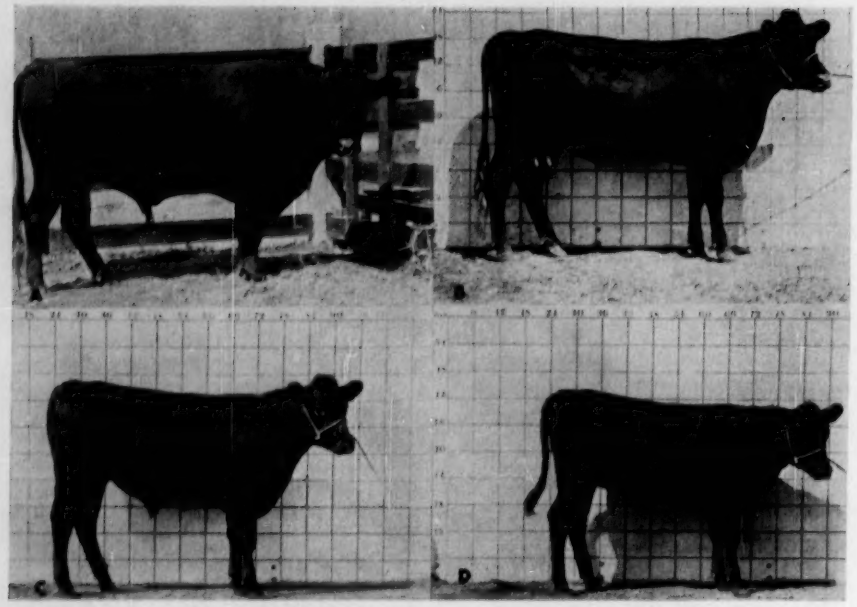

PARENTAL STOCK AND CROSSBRED CALVES

Figure 8

A—Red Dane bull (D504) at two years and eight months of age. One of the two Red Dane bulls used in the crossbreeding experiments. *B*—Red Poll cow (Chinsegut Victoria 82958) at two years and nine months of age. This cow was used in the crossbreeding experiments with the Red Dane bulls. She produced 5436 pounds of milk and 211 pounds of butterfat, in 305 days, her first lactation. *C*—Red Dane × Red Poll steer at 11½ months of age. *D*—Red Dane × Red Poll heifer at nine months of age.

ing) produced an average of 5591.2 pounds of milk and 228.4 pounds of butterfat in the 305-day test as first calf heifers. These figures were lower than the average produced by their crossbred dams as first calf heifers by 646.6 pounds of milk and 39.8 pounds of butterfat.

In the beef production phase, records were completed on 22 Red Poll, 19 F_1 crossbred Red Dane × Red Poll, and 14 head of back-cross (¾ Red Poll + ¼ Red Dane) steers. These steers were individually fed a limited ration of mixed grain, citrus pulp and hay during the fattening period from 450 to 850 pounds. Average birthweights for each of the 3 above-named groups were 75.2 pounds, 78.3 and 79.8 pounds, respectively. The group of crossbred steers attained an average weight of 450 pounds at an average of 255 days, the back-cross group at 273 days, and Red Poll steers at 304 days. The crossbred steers averaged 1.45 pounds a head daily during the fattening period from 450 pounds to 850 pounds liveweight, and led the back-cross steers by .015 pound a head daily and the Red Poll steers by .03 pound a head daily during the period, virtually no difference. However, in terms of grade standard prior to December 29, 1950 the Red Poll steers yielded carcasses averaging "Good," whereas those of the other two groups each averaged "High Medium," a difference of ⅔ of a grade in favor of the Red Poll steers. Dressing percentages for the three groups were 58.34, 57.41, and 58.42, respectively. In respect to efficiency (pounds of gain per 100 pounds of total

digestible nutrients consumed during the fattening period) the averages for the 3 groups were: Red Poll 14.63; crossbred Red Dane × Red Poll 14.67; and back-cross (¾ Red Poll + ¼ Red Dane) 13.48.

The first crossbred steers were contemporary with the first of the Red Poll steers that were on the feeding test. The back-cross steers were on feed during the years the final group of Red Poll steers was tested. Practically all of the steers of the three groups were on feed during the hot weather months of the year which may possibly have had some effect on their feed consumption and rate of gain.

It is difficult to draw definite conclusions because of the relatively small number of animals and particularly the very limited foundation of Red Dane cattle involved. However, under the conditions of the experiment the crossing appeared to improve the milk production and possibly the butterfat production of the crossbreds in comparison with the Red Polls and to have lowered the grade of the crossbred steers but not the growth or efficiency of feed utilization of either the crossbred steers or heifers in comparison with purebred Red Poll animals. In fact, the crossbred heifers appear to have a little advantage in rate and economy of growth over the purebred Red Polls. In general, the back-cross animals did not do quite as well as the first-cross Red Dane × Red Polls.

These results are from only one station and that in the extreme Southern part of the country, therefore, the results might not be the same as if the cross were made in another situation such as further north.

◆

A MOSAIC AFFECTING THE DOWN OF THE CHICK

KENNETH GOODWIN*

A CHICK with abnormal down covering most of one side of its body was observed recently among a group of newly-hatched pure White Leghorns (Figure 9). It was apparent that this was a sectorial mosaic of the type known as "half and half." While mosaics of various kinds have been reported in several species of domestic birds including the chicken, turkey, pigeon, and budgerigar[3], these have had their effect upon the skin or plumage color of the adult. It is thought that this is the first report of a mosaic involving the structure of the down.

The abnormal down covered only the left side of the body, with a sharp and distinct mid-ventral line of demarcation from beak to vent. Dorsally, the line separating normal from abnormal was distinct in the head region, but obscure over the back. The left wing and leg were covered with the abnormal down.

The defective down was coarse and sticky (as though the chick had not dried off and fluffed out), and was somewhat lighter in color than the normal down. When examined under magnification the individual plumules were shorter than normal, and had not unfolded from the sheath or spread out in the usual way. A comparison of the length of normal and abnormal down was obtained in a manner similar to that described by Hutt[6]. Instead of projecting the plumules on a screen, however, they were measured to the nearest 0.5 mm. directly under a dissecting micro-

*Kimber Farms, Inc., Niles, California.

scope. Samples of down were taken from five normal areas and five corresponding abnormal areas, with approximately ten plumules measured from each. The mean values, in millimeters, in the various regions are as follows:

	Normal	*Abnormal*
Head, top	11.8	7.3
Back	12.1	11.5
Abdomen	12.4	8.0
Leg, lateral surface	11.4	8.8
Wing, dorsal surface	15.7	11.1

Except in the case of the sample from the back, where no clear-cut line of demarcation was visible, each difference between pairs of regions was significant at the 1 percent level (*t* test).

Unfortunately, the chick was spraddle-legged when hatched and experienced considerable difficulty in moving around to eat and drink. It died at eight days of age, and at autopsy proved to be a male. The primary wing feathers appeared to be growing normally. The testes were grossly normal, there was no indication of any asymmetry in body size, and no attempt was made to obtain skeletal measurements.

The possible explanations for such mosaics have been discussed by Hollander[3], Hutt[4], and Cock[2]. A mutation having an effect similar to the one herein described, but not confined to a particular region of the body, has been described by Hutt and Long[6] and Buss, Bohren and Warren[1]. The present case, in the absence of any evidence of asymmetry in size, might be accounted for simply by a somatic mutation of a gene affecting the formation of the down.

HALF AND HALF MOSAIC

Figure 9

Ventral surface of White Leghorn chick with sectorial mosaic involving structure of the down. Defective down covered left half of the body; the mid-ventral line of demarcation is clearly seen in the figure.

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A BEAN INTERSPECIFIC HYBRID

SHIGEMI HONMA*

AS part of a program to incorporate common bean blight resistance into *Phaseolus vulgaris* L., the common bean, hybridization of this species with *P. acutifolius* Gray, a tepary bean, was investigated.

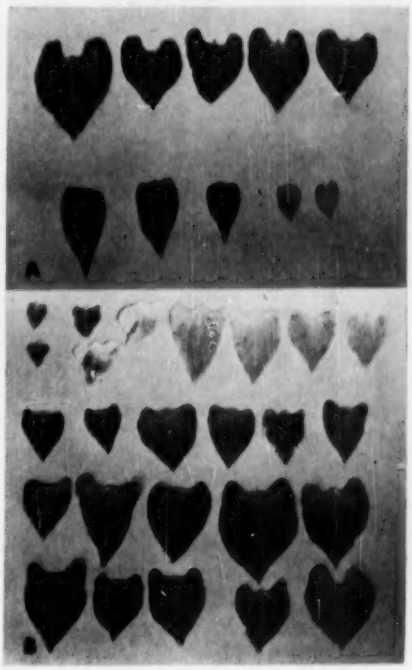
Interspecific hybridization in the genus *Phaseolus* is not new; Mendel[7] reported the crossing of *P. vulgaris* and *P. multiflorus* Lam., a runner bean. Lamprecht[5] has reviewed and described other studies of hybridization involving these two species. A cross between the species *P. vulgaris* and *P. mungo* L., the urd bean, has been reported by Strand[9]. Lorz[6] described a cross between *P. lunatus* L., the lima bean, and *P. polystachyus* (L.) B.S.P., a thicket bean. However, the cross *P. vulgaris* × *P. acutifolius* has not previously been reported to the writer's knowledge.

This paper summarizes the hybridization technique, the results of hybridization, and the characteristics in the F_3 generation.

Parental Description

The varieties Great Northern (*P. vulgaris*) and Tepary 4 (*P. acutifolius*) were used for the investigation. The tepary bean has been reported by Schuster[8] and other workers to be resistant to the common bean blight, *Xanthomonas phaseoli* (E.F.Sm.) Dows., while the Great Northern is considered to be susceptible.

Several distinctly different morphological characters of the two species are listed in Table I. The measurements of mature plant parts were taken from plants grown in the greenhouse. The flowers of the common bean are larger than those of tepary. The primary leaves of the two species are simple but the leaf base of *P. acutifolius* is truncate while that of *P. vulgaris* is cordate (Figure 10*A*). The secondary leaves of *P. acutifolius* have apices that are more acute than those of *P. vulgaris* (Figure 11). The parental differences recorded are similar to those described by Freeman[2] for these species.



PRIMARY LEAF BASE

Figure 10

A—The top row shows *Phaseolus vulgaris*, which is cordate. The bottom row shows the truncate type of *P. acutifolius*. *B*—Shows the primary leaf base segregation in the F_3 generation.

Hybridization Technique

The pollination method used was a modification of that described by Wade[10].

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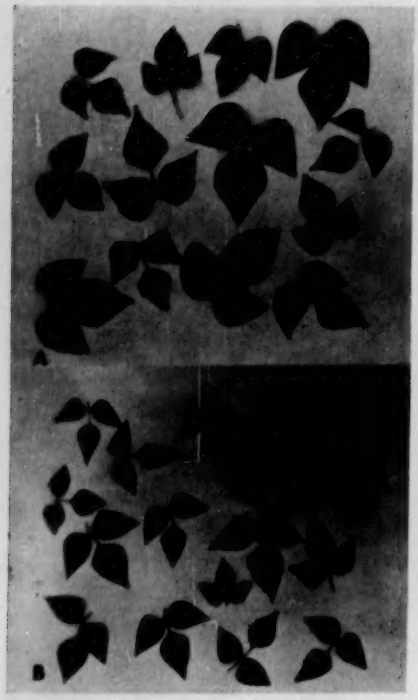


TYPICAL MATURE LEAVES

Figure 11

A—Phaseolus vulgaris. B—P. acutifolius. The secondary leaves of *P. acutifolius* have apices that are more acute than those of *P. vulgaris.*

MATURE HYBRID LEAVES

Figure 12

A—Shows the mature leaves of the interspecific hybrid. *B*—Mature leaf segregation in the F_2 generation.

Flowers of the female parent, *P. vulgaris,* were emasculated as follows: The standard was opened by scratching the suture with a sharp pointed tweezer. One of the wings was then clipped at the base to allow a better view of the keel. Small portions of the keel were then removed by pricking the bent portion near the apex, which minimized breakage and injury of the curled and brittle style. After removing the stamens, the stigmatic surface and style were examined with a 15× hand lens for pollen and possible injury.

Pollen-laden stigmas of the male parent, *P. acutifolius* were used as pollen bearers. These stigmas were obtained by bending the wings of the flower toward the pedicel, which forced the stigma to protrude from the aperture of the keel.

Pollen transfer was accomplished by brushing the "male" stigma carrying pollen on the stigmatic surface of the female flower. The "male" stigma was then left adhering to the newly pollinated stigma of the female parent. In order to maintain a high humidity about the stigma, the standard from the "male" flower was crushed in the palm of the hand with the thumb, moistened with saliva, and placed over the closed "female" standard. Excess flowers were removed to eliminate competition. During the crossing period, high humidity was maintained in the greenhouse.

Most of the flowers set pods, but all failed to reach maturity. Embryo abor-

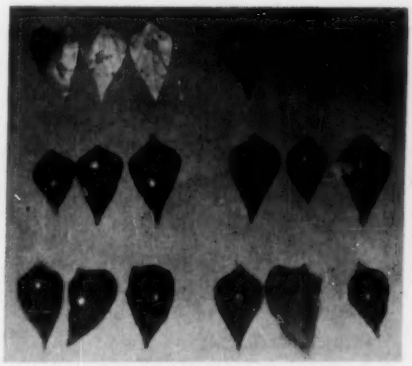
tion occurred within three to 24 days following pollination. Hormone applications suggested by Wester and Marth[11] to increase seed set did not increase the number of pods set nor did they delay the time of abortion. To obtain F_1 plants from the cross, embryos were excised and cultured *in vitro*[3]. From several hundred embryos, only four mature plants were obtained. The reciprocal cross using *P. acutifolius* as the female parent did not form pods or seeds. When intraspecific F_1 plants of *P. acutifolius* were used as female parents a few pods set but the embryos failed to attain sufficient size for culture *in vitro*.

Testing for Resistance

Inoculations were made in the greenhouse when the first set of secondary leaves were expanded. The underside of the terminal leaflet of the trifoilate of each plant was inoculated, using a modification of a method described by Schuster.[8] A shield with a six mm. hole placed against the leaflet and sprayed with the bacterial suspension was found to give a uniform inoculation.

Symptoms of blight infection were evident 10 to 14 days after inoculation. First, a thin border of water-soaked area appeared around the inoculated area. This was followed by chlorosis. The chlorotic area grew larger until the entire leaflet turned yellow and dropped off.

A classification of plant reaction was made at the time the entire leaflet of the susceptible lines and controls became yellow. Individual plants were classified as 0, 1, 2, 3, 4, and 5 reaction types. Type 0 showed no reaction around the tissue killed by inoculation; 1 included types with a narrow yellow ring around the inoculated area; 2 produced a slightly larger chlorotic band; 3 a larger band than 2; 4 being larger than 3 with irregular spreading; and in 5, yellowing of the entire leaflet. These reaction types are illustrated in Figure 13.



TYPES OF BLIGHT REACTION

Figure 13

Top row: left, type 5; right, type 4. Middle row: left, type 3; right, type 2. Bottom row: left, type 1; right, type 0.

Characteristics of the Hybrid

The interspecific hybrid plants were shorter than either parent. Foliar characteristics in the young plants resembled the tepary parent, but later stages of vegetative growth appeared more like *P. vulgaris* (Figure 12*A*). The inflorescence and pods were also similar to those of *P. vulgaris*. The flowers were self-fertile and produced pods with viable seeds. Seed number per pod were comparable to that of the *P. vulgaris* parent.

The diploid chromosome number of the two species has been reported as 22.[1] Root-tip preparations of the hybrid and parents did not suggest any marked morphological differences between the chromosome complements. A similar report on the chromosome morphology of these two species was made by Karpechenko.[4]

TABLE I. Parental averages and F_2 segregate averages and range for certain botanical characters used for comparison

Botanical Character	*Phaseolus acutifolius**	*Phaseolus vulgaris**	F_2 Segregation†		P‡
	avg.	avg.	avg.	range	
Seed size	.11 ± .01 gm.	.34 ± .02 gm.	.32 ± .05 gm.	.20 — .47 gm.	.001
Petiole length (primary leaves)	4.1 ± .2 mm.	41.9 ± 3.9 mm.	35.2 ± 5.1 mm.	9 — 53 mm.	.17
Length of primary leaves	46.3 ± 4.4 mm.	72.6 ± 5.2 mm.	75.7 ± 7.1 mm.	66 — 96 mm.	.73
Width of primary leaves	30.3 ± 3.7 mm.	52.8 ± 4.7 mm.	48.3 ± 5.5 mm.	41 — 63 mm.	.53
Ratio: length/width, primary leaves	1.52 ± .15	1.38 ± .09	1.57 ± .09	1.38 — 1.71	.11
Length of terminal leaves	51.5 ± 4.9 mm.	83.7 ± 6.2 mm.	87.2 ± 9.9 mm.	73 — 99 mm.	.76
Width of terminal leaves	32.0 ± 1.3 mm.	58.4 ± 3.4 mm.	52.9 ± 6.3	42 — 64 mm.	.44
Ratio: length/width, terminal leaves	1.62 ± .07	1.41 ± .04	1.65 ± .13	1.51 — 1.81	.07

* Measurements from 25 parental plants of each species.
† Measurements from 250 plants sampled from the F_2 segregating population.
‡ P is probability of true difference between the F_2 hybrids and *P. vulgaris*.

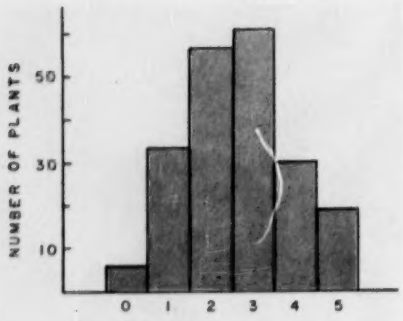


Figure 14

Distribution of 206 plants sampled from the F_2 population for blight reaction.

Characteristics of the Segregating Generation

Since only a limited number of seeds were obtained from the F_1 plants, blight reaction and botanical differences were studied in the F_2 generation. The parents and segregates were grown in the greenhouse.

Measurements of mature plant parts were made and the probability of true difference between the F_2 hybrids and *P. vulgaris* are shown in Table I. A definite difference in seed size was noted between the F_2 hybrids and the *P. vulgaris* parent. For the characters, petiole length and ratios of length/width of the primary and terminal leaves, the P values suggest a difference between the F_2 hybrids and *P. vulgaris*.

In general the segregates were larger than the *P. acutifolius* parent. The primary leaf bases ranged from truncate to cordate in shape (Figure 10*B*). Variations in mature leaf shapes were also present, (Figure 12*B*).

The plants of the F_2 population showed varying degrees of resistance to common blight. Distribution of reaction types sampled from the segregating population is shown in Figure 14.

Discussion

The data and figures demonstrate that a cross between the two species, *P. vulgaris* and *P. acutifolius*, was secured. The variation of characters in the F_2 generation suggest a quantitative type of inheritance. From the data as shown in Table I, there were measurements of the F_2 segregates which exceeded that of *P. vulgaris*, the larger parent, whereas none of the measurements of *P. acutifolius* were recovered. A probable explanation for the appearance of segregants larger than *P. vulgaris*, but none as small as *P. acutifolius*, may be the result of hybrid vigor.

Summary

Four plants were produced from the cross, *Phaseolus vulgaris* × *P. acutifolius*. The hybrids appeared more like the *P. vulgaris* parent. Chromosome smears of root tips showed no morphological differences between the complements of the hybrids and the parents. Common blight reaction and botanical characters which differed distinctly between the parents were found to be quantitatively inherited in the F_2 generation.

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AN EXTREME NON-AGOUTI MUTANT IN THE MOUSE

W. F. Hollander and John W. Gowen*

AGOUTI is a term used to designate the black and yellow banding of the hair commonly seen in wild rodents. In the mouse (*Mus musculus*), five distinct alleles affecting the hair patterns are listed by Grüneberg[1]:

- A^y "yellow"; lethal if homozygous; heterozygote becomes obese.
- A^w "white-bellied" or yellow-bellied agouti.
- A^+ uniformly agouti, the usual wild type.
- a^t "black-and-tan"; belly yellowish, rest of body black.
- a "non-agouti"; black.

The dominance seriation is roughly in descending order, but the yellowish belly effect of a^t is plain in A^+ a^t mice. A more logical summary of the dominance relations would be that the production of less black is dominant to the production of more. The black-and-tan factor produces more dorsally, less ventrally, hence may be considered recessive dorsally and dominant ventrally.

Wallace[3] has discussed the possibility that these factors may be pseudo-alleles, i.e., very closely linked, capable of crossing-over and independent mutation. A decision between the two alternatives was not possible on the basis of the considerable data in the literature. However, the theory of two loci was thought to have "slightly more in its favour than the single-locus theory". A slightly different set of symbols was used: A^Y, A^L, A, a^t and a.

Apparently it has always been assumed that the ordinary black (non-agouti) allele is the ultimate possible recessive member of this series. But examination of non-agouti mice on any stock always reveals some yellow hair in the ears, the perineal region, and around the nipple areolae. A blacker type is therefore conceivable. The present paper reports such a type found as a mutant.

The circumstances of discovery of this "extreme non-agouti" are out of the ordinary and require explanation. X-rays (500r) were delivered to the pelvic region of adult male mice of our S strain (albino, resistant to *Salmonella typhimurium*). These males were then mated for two weeks with females of the P stock. The P stock is a well-known multiple-recessive tester, having five recessive mutant markers, *aa, bb, pp, dd, sese.* The S strain has none of these markers; all progeny of the cross are expected therefore to be wild-type. Among over 1500 F_1 mice, a single black one was found. It had three sibs, all wild-type.

The black F_1 mouse was a typical non-agouti male, quite vigorous and healthy. The possibility of a deficiency for the "A-locus" was considered, and matings were made to test this idea. The mates chosen for him were yellows, of genotype A^yA^+. These females were vigorous and young, but only three litters were produced, totalling eight young. Subsequently the male failed to impregnate any females. Such impairment of fertility is not uncommon in progeny of X-rayed mice; its cause is ordinarily unknown, but it has been attributed to chromosome damage, such as translocations.

Five of the eight young were raised to maturity; all were males, four yellow and one wild-type. The next step in isolating the alleles was to cross each of these males with a type lower in the series, especially with black-and-tan. From such a mating only black-and-tan progeny were to be saved, for mating within the family. In each family line, only one of the two homologues present in the original black mouse could segre-

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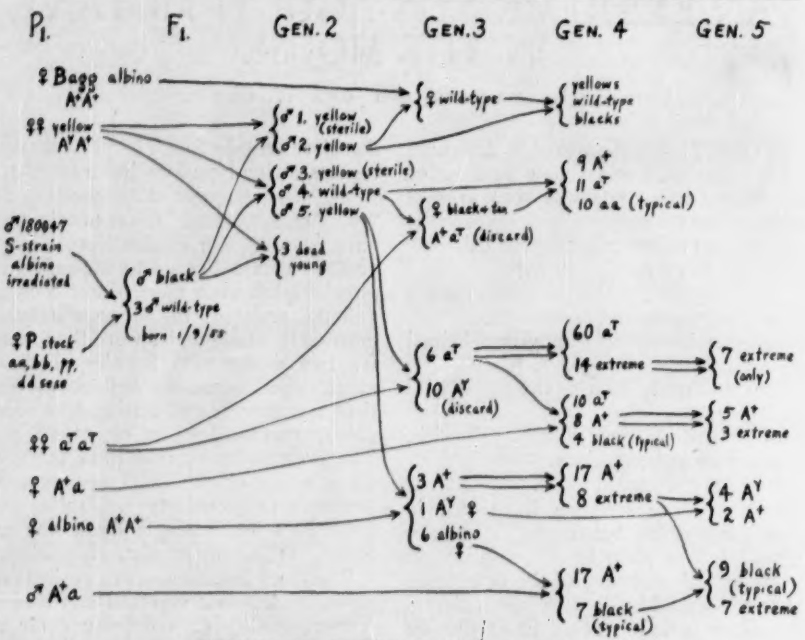


ORIGIN OF EXTREME NON-AGOUTI

Figure 15

Pedigree chart showing the origin of the extreme non-agouti mutant and results of early test matings. Brackets enclose sibs. In generation 2, the five sons of the F_1 black are given identification numbers. Other numbers in the chart are counts of mice of the types indicated.

gate—the *a* from the P stock or the presumed mutant from the S (X-rayed) male. (See Figure 15.)

Unfortunately two of the five males produced no progeny at all, despite good health and plenty of females. Subsequent dissection of these males and also the original black mutant revealed relatively few sperms in the semen, and the testes were rather small. The remaining three males were completely fertile.

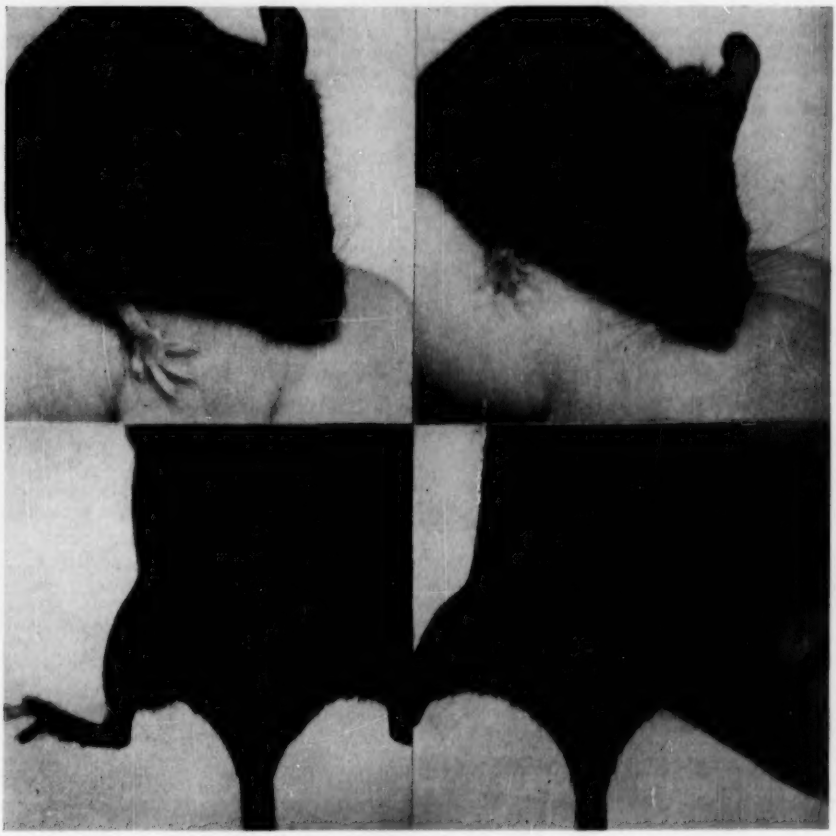
All three of these males yielded some black descendants, showing that if a deficiency of the A-locus were involved, it was not lethal. In one of these families the blacks were noticed however, all to be extreme, with no yellow hairs, (see Figure 16). The extreme blacks have been healthy and vigorous, apparently as viable as the ordinary type. Their fertility has been high.

The breeding tests shown in Figure 15, as well as additional data, allow the following conclusion: the extreme non-agouti type acts as a recessive to all other types in the *A* series, and segregates as an allele (or pseudo-allele?) to all.

Classification of extreme non-agouti in combination with other markers such as brown, dilute, or pink-eyed dilute is best at the age of about two weeks, before the ears expand greatly. The most useful combinations seem to be those permitting black pigmentation, such as belted, piebald, or dominant spotting.

Discussion

It is clear that the extreme non-agouti type is a new allele in the *A*-series, rather than an incidentally incorporated modifier. Whether it was produced by the X-ray treatment is less clear, but in no such experiment can the



NON-AGOUTI—ORDINARY AND EXTREME
Figure 16

Comparison of ordinary non-agouti black mouse (left), with extreme non-agouti black (right). Both are females. Note the yellow hair in the ears and around the perineum and nipples of the ordinary black mouse.

evidence be more than circumstantial. Russell[2] found no mutations at this locus, in a somewhat similar experiment, among nearly 50,000 progeny, as well as a large number of controls.

Of course, many of the "mutations" reported in the literature may have been crossovers of some sort, as argued by Wallace[3]. But such a basis cannot be invoked in the present case, since the stocks crossed were not heterozygous at this locus. Unequal crossing-over is a different matter; this would in effect produce a deficiency for the locus.

The existence of the new allele complicates the argument for compound structure of the "*A*-locus." Wallace did not include the yellow allele in the formulations since Fisher had suggested to her that yellow might be a deletion rather than a gene effect. If extreme non-agouti is considered a deficiency, yellow could hardly be considered such also, and vice versa. Then three allelic point mutants would exist for one of the two presumed components unless one assumes a tripartite locus.

Although the compound-locus idea is attractive, it seems necessary for further experimental attack to be made. Perhaps the extreme non-agouti will serve as the best tester type in such experiments. Meanwhile it presents a problem in symbolization. The easiest solution is simply to assign it the symbol a^e. However, if changing of symbols is to be everybody's privilege, as Wallace has done, the

following revision should be considered: A^y and A^L (in agreement with Wallace), $A+$, a^T, a^1 ("primary non-agouti"), and a (extreme non-agouti). This system has the merit of showing, by capitalization, dominance relations between the wild type and each other allele.

Summary

A completely black mutant type in the mouse is reported, showing no yellow hair in the ears etc. such as is found in ordinary black mice. The mutant is shown to be a sixth allele, a^s, in the "A" color-pattern series, and the most recessive of all. It originated in a mouse treated with 500r of X-rays. The question of composite structure of this locus is discussed.

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◆

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Application blanks may be obtained from Dr. Dorothy Quiggle, Petroleum Refining Laboratory, The Pennsylvania State University, University Park, Pennsylvania. Announcement of the award will be made early in March 1957.

◆

National Academy of Sciences-National Research Council Postdoctoral Resident Research Associateships

The National Academy of Sciences-National Research Council has announced that its programs of Postdoctoral Resident Research Associateships will again be offered for the 1957-1958 academic year at the Argonne National Laboratory, the National Bureau of Standards, and the Naval Research Laboratory. The NAS-NRC also announces the inauguration of a new associateship program at the Oak Ridge National Laboratory.

These Postdoctoral Resident Research Associateships have been established to provide young scientists of unusual ability and promise an opportunity for advanced training in basic research in a variety of fields. The most modern facilities are available in the general areas of biology, ceramics, chemistry, electronics, mathematics, metallurgy, and physics Examples of some of the more specific fields within these areas are as follows: radio astronomy, statistics and mathematical biology, computer design, radiation and high temparature chemistry, microwave component theory, semiconductors, numerical analysis, liquod metals, low temperature, solid state and reactor physics. In addition to the above, research in visual psychophysics and engineering psychology is also available.

Applicans must be citizens of the United States. They must produce evidence of training in one of the listed fields equivalent to that represented by the Ph.D. or Sc.D. degree and must have demonstrated superior ability for creative research. Stipends are up to $7035 a year.

Further information and application materials may be secured by writing to the Fellowship Office, National Academy of Science-National Research Council, 2101 Constitution Avenue, N.W., Washington 25, D. C. In order to be considered for awards for the academic year 1957-1958, applications must be filed at the Fellowship Office on or before *January 11, 1957*. Awards will be made about April 1, 1957.

HEREDITARY SPHEROCYTOSIS IN PEROMYSCUS

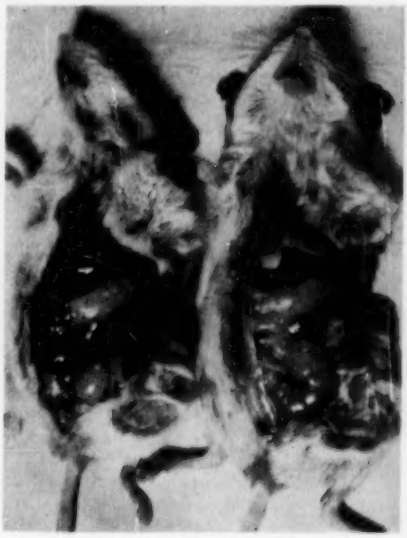
I. Genetic Studies

R. R. Huestis, Ruth S. Anderson and Arno G. Motulsky*

A GENETIC red cell defect in the deer mouse was discovered and previously reported as inherited neonatal jaundice[5]. Further investigations showed that the various pathologic and hematologic characteristics of the syndrome closely resembled those of human hereditary spherocytosis[6]. These features produced a hemolytic syndrome associated with spherocytosis and splenomegaly. A series of studies was therefore undertaken to compare the murine syndrome with its human counterpart. The present article presents the results of the genetic investigations of murine hereditary spherocytosis.

Materials and Methods

The colony in which the spherocytic segregants were found is made up of the cage-born descendants of mice trapped in Oregon and California together with some descendants of individuals obtained some years ago from Dr. Lee R. Dice. Pregnant females were checked daily or more frequently to make possible inspection of young mice within the first 24 postnatal hours. Affected newborn mice were identified by the presence of jaundice which was usually followed by pallor. Visible differences between the phenotypes disappeared rapidly after the first or second day of life. Postnatal identification was routinely checked by the breeding record and post mortem by the weight and appearance of the spleen (Figure 17). These tests showed that immediate postnatal phenotypic identification was reasonably but not completely accurate since an occasional mouse was difficult to classify at birth. The mice included in the tabulations involved for the most part animals that were checked by postnatal as well as adult criteria.



SPHEROCYTIC AND NORMAL

Figure 17

A spherocytic mouse showing an enlarged spleen which lies diagonally below the greater curvature of the stomach and an unaffected mouse with a normal spleen in the same position.

When first discovered, the gene inci-

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dence of hereditary spherocytosis in the colony was already high. Therefore, only trapped wild mice and their cage-born offspring were used for the wild type control series. This control group has not produced jaundiced or splenomegalic progeny in any mating.

Results

Breeding Tests

Affected animals were bred with each other and with normal wild type and heterozygous animals. Table I presents the classes of offspring obtained from these matings. These results suggest that the spherocytic syndrome in mice is transmitted by a single recessive autosomal gene.

Table II presents the recombination ratios produced by mating flexed-tailed spherocytic males with females which had one wild type and one flexed-tailed spherocytic parent. The genes, if linked in these females, would be in the coupling phase. The data indicate that the genes for flexed tail and spherocytosis recombine independently. Inferentially, the spherocytic syndrome is therefore not linked with albinism, pink eye, or silver pelage, all of which have been reported to be linked with each other[4,9].

Genetic Factors Producing Variability of Jaundice

During the first postnatal day some affected mice became very jaundiced while others with the spherocytic syndrome may not differ markedly from non-affected mice of similar age. This difference is particularly striking when it is present in the same brood. The possibility that genetic factors caused this variability in icterus was tested by mating mice which had been very jaundiced at birth with each other and with other affected and heterozygous animals. The results are presented in Table III and show a high correlation in icteric appearance at birth between parents and young.

These results make it appear highly probable that the differences in neonatal jaundice are to a considerable extent caused by genetic factors. Further studies will have to decide whether severe icterus is caused by a more severe hemolytic syndrome or may be due to genetic inferiority of the liver in removing bilirubin efficiently.

Viability of Spherocytic Mice

There is some indirect evidence that spherocytic mice are not at any great selective disadvantage in comparison with nonaffected mice reared in captivity. In 100 litters each produced by spherocytic females, heterozygous females, and other females chosen at random for comparison, the average number of young per litter, with standard errors, was $3.84 \pm .14$, $3.94 \pm .14$ and $3.74 \pm .11$ respectively. The differences are not significant.

The gene for spherocytosis had reached an incidence of about 0.3 at the time the neonatal jaundice was first observed in the colony. This inference is based upon the breeding record of 42 mice chosen at random, 22 of which produced jaundiced young when mated with known affected mice or heterozygotes. This gene incidence and the equal number of affected and nonaffected young found in the test-cross progenies are evidence of successful compensation for the red cell defect in the spherocytic mice.

TABLE II. A test for linkage of spherocytosis and flexed tail in heterozygous females which received both genes from one parent. Male parents flexed and spherocytic

Young mice	Observed	Expected if not linked	χ^2	P
flexed spherocytic	23	25		
neither flexed nor spherocytic	28	25		
flexed only	20	25		
only spherocytic	29	25		
n	100	100	2.16	.70 – .50

TABLE III. The proportion of very yellow spherocytic young produced by parents with different intensity of jaundice after birth

Parents	Offspring: Very yellow	Offspring: Not very yellow	n	% vy
Both very yellow	20	11	31	64.5
One very yellow	25	91	116	21.6
None very yellow	33	659	692	4.8

TABLE I. Dominance of the normal allele + and segregation of the gene *sp*, for spherocytosis, in different matings

Parents	Young	Observed	Expected	n	χ^2	P
sp/sp × *sp/sp*	affected	128	127			
	normal	0	1	128	1.003	.50 – .10
+/+ × *sp/sp* &	affected	0	1			
sp/sp × +/+	normal	140	139	140	1.006	.50 – .30
+/*sp* × *sp/sp* &	affected	144	343			
sp/sp × +/*sp*	normal	342	343	686	0.003	.95 – .70

Discussion

This syndrome in Peromyscus is of marked interest because of its close similarity to that of human hereditary spherocytosis. Detailed hematologic studies on the murine syndrome have been performed and will be reported elsewhere. Table IV summarizes the comparison of the various clinical, pathologic and hematologic features of hereditary spherocytosis in mouse and man. It should be noted that a basic lesion marked by the diminished life span of the spherocyte leads to the complete syndrome. All other pleiotropic effects of the gene can be related to this basic abnormality.

It has been shown that, analogous to the human situation, murine spherocytes are selectively trapped by the splenic pulp where they are destroyed. Any normal spleen is effective in removing spherocytes as demonstrated by the shortened survival of spherocytes in the circulation of normal and heterozygous mice. Splenectomy by removal of the splenic trap allows normal survival of spherocytes in spite of the persistence of the genetic defect and thereby cures the hemolytic process[6]. The murine syndrome in the adult mouse is not associated with significant anemia in spite of the shortened red cell survival. The bone marrow has been able to compensate for the shortened red cell survival by increasing its mass and output as witnessed by the marrow hyperplasia and reticulocytosis. Similar findings have been noted in man.[3, 10]

TABLE IV. A comparison of hereditary spherocytosis in mouse and man

	Mouse	Man
Genotype of affected subjects	Recessive homozygote	Dominant heterozygote
Fertility	Unimpaired	Unimpaired (shortage of affected siblings)
Spherocytosis	Present	Present
Increased osmotic fragility	Present	Present
Reticulocytosis	Present	Present
Normoblastic hyperplasia of bone marrow	Present	Present
Neonatal jaundice	Present	Rare
Neonatal anemia	Present	Rare
Adult anemia	Mild to none	Mild to none
Adult jaundice	Present	Present
Splenomegaly	Present	Present
Red cell congestion of splenic pulp	Present	Present
Increased incidence of gallstones	Yes	Yes
Crises	Not yet observed	Aregenerative or hemolytic
Results of splenectomy	Hemolysis markedly decreases	Clinical cure
Life span of spherocytes in normal circulation	Shortened	Shortened
Life span of spherocytes in splenomegalic circulation	Shortened	Shortened
Life span of spherocytes in splenectomized circulation	Normal	Normal
Life span of normal cells in affected splenomegalic circulation	Normal	Normal

It has been shown in this paper that hereditary spherocytosis in the mouse is caused by a gene the effect of which is only apparent in the homozygous state. In contrast, human hereditary spherocytosis is caused by a somewhat rare dominant gene so that the affected person is typically heterozygous. It has been suggested that the gene for human hereditary spherocytosis is lethal when homozygous[8], but Bernard *et al.*[1] report a case of a spherocytic father with a family of 13 spherocytic children, a possible case of homozygosity of the father. Ways in which the gene for spherocytosis may be identified in heterozygous mice are being investigated.[2]

The demonstration that the severity of the neonatal jaundice is under genetic control is of great interest. These findings suggest that genetic modifiers of unspecified nature may produce the variability of the syndrome in the mouse and, by inference, in man. It has been reported in careful human family studies that occasionally no evidence of hereditary spherocytosis was apparent in the parents of typical cases[8, 10]. In the absence of illegitimacy or mutation, low expressivity of the gene has been invoked in such instances. Recent human studies suggest that red cell survival tests may detect such "carriers."[7] Further investigation of the exact genetic and pathophysiologic nature of the modifiers producing variability of the syndrome are in progress and may shed further light on the human cases.

Summary

A hemolytic syndrome closely resembling human hereditary spherocytosis has been described in Peromyscus.

The syndrome is produced by a single autosomal recessive gene substitution. Affected animals are homozygous. Heterozygotic carriers cannot be distinguished from normal animals.

The gene for spherocytosis is probably not linked with albinism, pink eye, flexed-tail or silver pelage.

The various pleiotropic effects of the gene such as jaundice, splenomegaly and reticulocytosis are produced secondarily by the basic gene defect that produces spherocytosis.

Variability in the severity of jaundice at birth was shown to be probably due to the influence of genetic modifiers.

The implications of these various findings for human hereditary spherocytosis are discussed.

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NO LYSENKOISM FOR HUMANS!

This is the reply received by a Belfast student to a letter he sent officials in Moscow, inquiring as to the official Russian Position regarding the relation of the Nature-Nurture issue to such human problems as education:

THE EMBASSY OF THE
U.S.S.R.
13, Kensington Palace
London W. 8

14th June 1956

Dear Sir,

In reply to your letter, I wish to inform you that theory of heredity and environment is applied in our country only to plants and cattle breeding in agriculture, and has nothing in common with problems of education.

Herewith I have the pleasure of forwarding you some booklets, which might be of interest to you.

Yours sincerely,
(signature undeciphered)
Secretary

◆

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COLCHICINE-INDUCED TRUE-BREEDING CHIMERAL SECTORS IN FLAX

V. A. Dirks, J. G. Ross and D. D. Harpstead*

THE induction by colchicine treatment of homozygous diploid variants in sorghum without change in chromosome number has been reported by Franzke and Ross.[5] Ross, Franzke and Schuh[8] have demonstrated that agronomically important characteristics in sorghum may be changed as a result of treatment and that the progeny of the changed plants breed true in the majority of cases. As part of a program to investigate the universality of this phenomenon in other biological material, seedlings of flax, soybeans, corn and wheat have been treated with colchicine in the same manner at the South Dakota Station. This study is concerned with the results of treatment of F_1 seedlings of crosses between varieties of flax containing gene markers to permit the detection of somatic segregation.[7]

Materials and Methods

The epicotyls of F_1 seedlings from eleven different flax crosses were coated with 0.5 percent colchicine in lanolin as soon as the cotyledons opened. The treated epicotyls formed typical c-tumors, and subsequently new shoots arose from these growths. Since Franzke and Ross[5] had noted chimeral effects in sorghum, seed from each of the main branches arising from the c-tumor was kept separate during harvest to permit the identification of possible chimeras. The progenies of the branches were grown in individual progeny rows in 1952 and classified for flower color, rust resistance and other obvious characteristics. Promising material was advanced to yield tests, consisting of four-row rod-row plots in three replications.

Surplus F_1 seed of a series of crosses made as part of a flax improvement program was used in this study. Some of these crosses involved a wide range of characters, which would permit recognition of somatic segregation. This report is primarily concerned with the progeny of two of these crosses: (a) Crystal (C.I. 982) × B-5128 (C.I. 980); (b) Crystal (C.I. 982) × Can. 3855B. Crystal is yellow-seeded, white-flowered, tall and wilt resistant, and carries the *L* factor for rust resistance.[8] B-5128 is brown-seeded, blue-flowered, tall, and moderately wilt resistant, and has the N^4 gene for rust resistance. Can. 3855B is a brown-seeded, blue-flowered type with the *L* factor for rust resistance. Brown seed coat is dominant to yellow; blue flowers are dominant to white; the genes for seed-coat color and for flower color are linked. Plants with either the N^4 or *L* gene or both are resistant to the Race 180 of *Melampsora lini* (Pers.) Lev. prevalent in the Brookings area in 1953, while plants without at least one of these genes are susceptible.

Observations and Discussion

When treated plants were harvested it was noted that in one F_1 plant of the cross Crystal × B-5128, branches differed in the color of seed produced. Branches 1, 2 and 3 had only brown seed; branches 4 and 5, only yellow seed; branch 6, a mixture of yellow and brown seed. Seed harvested from each of these branches is shown in Figure 18, arranged around seeds from an untreated F_1 plant. Seed from the sixth branch of the original plant was separated into yellow (6a) and brown (6b) fractions. In 1952, each lot of seed from the separated fractions and from each of the first five branches produced F_2 plants uniform within each lot for seed color, flower color, height, general plant type, and rust resistance. The true-breeding nature of these progenies in the next three generations was established by observations at Brookings in 1953, 1954 and 1955 (Table I). Mean yields taken during these three years indicated that as a group, the brown-seeded branches significantly outyielded the yellow-seeded branches during this period. They out-performed the yellow

*Agronomy Department, South Dakota State College, Brookings, South Dakota. Journal Paper Number 343, South Dakota Agricultural Experiment Station, Brookings, South Dakota.

branches individually at the 5 percent level of significance except for branch 3. Except for the low yield of branch 3 in 1954, which might occur by chance in 1 out of 20 trials, very little variability was observed within either the brown-seeded lines, or within the yellow-seeded lines. The two groups differed strikingly in wilt susceptibility, as seen in Table II, where the 1954 wilt readings on the F_4 progeny from the original branches of the chimeral plant are reported for three locations: Ottawa, Ontario, Fargo, North Dakota and Brookings, South Dakota. Progeny of branches 1, 2 and 3 were much more resistant than those from branches 4 and 5 as illustrated in Figure 19.

Since yellow seed and white flowers are recessive, the chimeral sections of the treated F_1 showing these characteristics are homozygous. Similarly, the absence of white-flowered or yellow-seeded segregates in the progeny of the original branches 1, 2 and 3 indicates they were homozygous for the genes for brown seed and blue flowers. Ordinarily, an F_2 population of several hundred plants of a cross involving complementary factors for rust resistance ($LLnn \times llN^4N^4$) segregates 15:1, and in succeeding generations, the recessive class approaches a frequency of 25 percent. The complete absence of susceptible segregates in either the brown- or the yellow-seeded derivatives indicates that each sector of the F_1 plant was homozygous for LL or N^4N^4 or both. The appearance of recessive characteristics in branches of the treated F_1 plant, the absence of segregation in successive progenies of the different branches, the distinct separation between the brown- and the yellow-seeded groups, and the unusual degree of similarity between the branches of each group appear to provide definite evidence that the original treated F_1 plant must have been a chimera of two or more homozygous sectors.

A treated F_1 plant from the cross Crystal × Can. 3855B also appeared to be a chimera. Branches 2 and 3 produced uniformly brown-seeded, blue-flowered progenies, but those of branch 1 segregated for flower and seed color (Table III). In addition, the progeny of branch 2 seemed to have a very distinctly higher yield in 1953, a favorable flax year. Whatever the nature of that yield advantage, it was not expressed under the high temperatures of the 1954 and 1955 seasons.

Cytological examination* of the above progenies indicated no change in the chromosome number. This is in accord with observations where growing points treated with high concentrations of colchicine were found to yield diploid adventitious shoots[1].

The origin of these chimeral sectors homozygous for marker genes may be explained by the same mechanism that Franzke and Ross[6] proposed for the origin of homozygous diploid plants after colchicine treatment of sorghum. Reductional grouping of the chromosomes (as described by Huskins[7]) would segregate the marker genes while subsequent separation of the two-partite c-chromosomes would restore the diploid nature and establish homozygosity for these genes. A cell so formed could, because of positional or genotypical advantage, form a new growing point which would become one of the branches of the flax plant.

A different type of response to colchicine, nevertheless explainable by the above hypothesis, was noted in the progenies of branches of other treated F_1 plants. There were cases where apparent induced homozygosity for one gene was associated with segregation for an-

*This examination was made by G. F. Atkinson, supported by research grant P.H.S. C-2086 from the National Cancer Institute of the National Institutes of Health, Public Health Service.

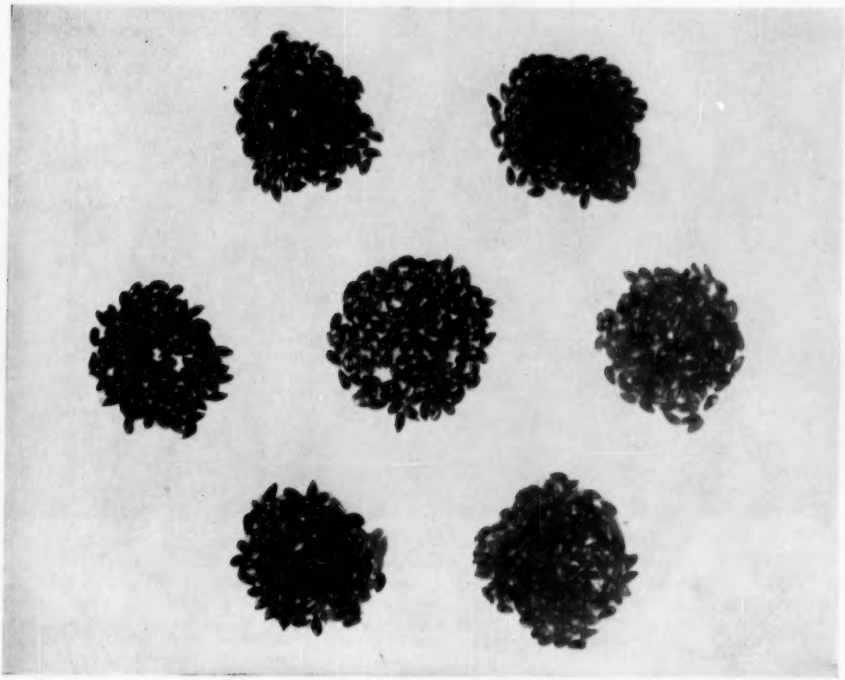
TABLE I. Progeny test of unselected F_3, F_4, and F_5 generations grown from separate branches of a colchicine-treated chimeral F_1 plant of the cross Crystal × B-5128 (Brookings, 1953-55)

Branch	Flower color	Seed color	Rust reaction	Yield in bushels per acre 1953	1954	1955	Average
1	blue	brown	Res.	22.2	18.0	5.3	15.2
2	blue	brown	Res.	23.4	16.0	5.4	14.9
3	blue	brown	Res.	21.3	15.2	4.9	13.8
4	white	yellow	Res.	18.4	17.3	3.2	13.0
5	white	yellow	Res.	19.7	16.9	2.9	13.2
6a	white	yellow	Res.	15.4	16.9	3.4	11.9
6b	blue	brown	Res.	20.3	18.4	8.6	15.8
Parents:							
Crystal	white	yellow	Res.	20.0	18.4	6.5	15.0
B-5128	blue	brown	Res.*	22.1	18.7	6.3	15.7
L.S.D.† at 5% level				2.1	2.4	1.8	1.2
Brown sector (Branches 1, 2, 3 and 6b)				21.5	17.0	6.5	15.1
Yellow sector (Branches 4, 5 and 6a)				17.8	17.2	3.2	12.7
Difference between sectors				3.7**	— 0.2	3.3**	2.4**

*B-5128 as a variety generally contains an admixture of rust-susceptible plants. Only resistant, typical plants were used in the crossing program.

†Least significant difference.

**Significant at the 1 percent level of probability.



SEED FROM SIX TREATED BRANCHES

Figure 18

Seed harvested from individual branches of a single colchicine-treated F_1 flax plant of the cross Crystal × B-5128. The branches were numbered consecutively, 1 to 6, beginning at left center and proceeding clockwise. Sample in the center is seed from an untreated F_1 plant of the same cross. Seed from each of the branches 1-5 of the treated plant bred true as did also the brown and yellow fractions in branch 6.

other gene. Such cases are presented in Table IV, and involve two other colchicine-treated F_1 plants of the cross Crystal × B-5128. In the first, F_2 progeny of branch 1 is segregating for rust resistance but not flower color. Branch 2 is homozygous for susceptibility to rust, although flower color appears to be segregating. Results from branches of plant 2 are less clear cut. Tests for heterogeneity indicate extremely little likelihood that branch 1 and 2 of plant 1 are drawn from the same population as far as genetic factors for rust resistance are concerned. Similarly, the five branches of plant 2 do not represent a single population with respect to segregation for flower color. These anomalous segregations could have resulted from small chimeral sectors that involved only sections of branches similar to the situation in the chimera of Table I, where two sectors were present in branch 6. Such branches could be mixtures of more than one kind of homozygous tissue arising from somatic reduction followed by doubling, homozygous plus heterogous tissue arising from somatic reduction plus mutation, or homozygous plus heterozygous tissue of the original F_1 constitution.

The hybrid nature of the material undoubtedly limited detection of mutagenic effects of colchicine, as reported for sorghums by Harpstead *et al.*[6] However, the appearance of rust susceptible recessive plants in progenies of crosses where both parents are homozygous for the same dominant factor for re-

TABLE II. Wilt disease ratings on F_2 progenies from separate branches of a colchicine-treated chimeral F_1 plant (Crystal × B-5128), 1954

Branch	Ottawa, Ont. percent infection	Fargo, N. D. (1-9)*	Brookings, S. D. (1-9)*
1	1.4	5	1
2	8.5	4	1
3	8.8	5	1
4	48.7	9	9
5	41.9	8	7

*1 being most free from disease.

WILT REACTION

Figure 19

Wilt reaction in the field of progenies of branches 1 to 5 (going from right to left) of the chimeral colchicine-treated F_1 flax plant of the cross Crystal × B-5128. The two yellow-seeded bulk progenies at the left are badly wilted, but the three brown-seeded bulk progenies are relatively resistant. (Brookings, 1954).

sistance indicates a mutation to the recessive condition. Such recessives appeared in the F_2 progenies of single branches of three treated plants of the cross Crystal (*LL*) × Can. 3855B (*LL*), at an approximate level of 25 percent. Similarly, two treated plants of Rocket (*LL*) × Marine (*LL*) gave rise to susceptible plants in the F_2 progeny of one branch per treated plant. The appearance of these in F_2 would have to be considered due to mutation to the recessive. The apparent limitation of the mutation to a sector of the plant would indicate its origin in the c-tumor state, apparently associated with the disturbances in genetic material occurring after colchicine treatment. A mutation to the dominant condition of a gene is suggested by the semi-dehiscent character of all the brown-seeded progenies of the chimeral plant reported in Table I. This would indicate that all the brown-seeded parts of that plant were from a single sector of the chimera. Semidehiscence is not found in either Crystal or B-4128 and has been reported as a genetic dominant[2].

Of the 162 F_1 flax plants surviving colchicine treatment, 10 plants were judged, on the basis of performance of subsequent generations, to have one or more branches constituted entirely from a single true-breeding chimeral sector. Results in Tables I, II and III are typical of these. Segregations of the kind reported in Table IV or possible gene mutations were involved in the progenies of 44 additional plants. This would indicate an observable genetic response to colchicine in 31 percent of the plants studied.

TABLE III. Progeny test of unselected F_3, F_4 and F_5 generations of progeny of separate branches of a colchicine-treated plant of the cross Crystal × Can. 3855B, (Brookings 1953-55)

Branch	Flower color	Seed color	Blooming date	Rust reaction	Yield in bushels per acre 1953	1954	1955	Average
1	seg.	seg.	6-10	Res.	21.1	16.6	7.7	15.1
2	blue	brown	6-10	Res.	25.5	16.5	6.7	16.2
3	blue	brown	6-10	Res.	20.2	18.2	8.4	15.6
Parents:								
Crystal	white	yellow	7-2	Res.	20.0	18.4	6.5	15.0
Can. 3855B	blue	brown	6-25	Res.	14.9	21.5	11.4	16.0
L.S.D.* at 5% level					2.1	2.4	1.8	1.2

*Least significant difference

In sorghum, the occurrence of true-breeding variants after colchicine treatment was originally explained by an hypothesis which assumed that ancestral genes in a putative allopolyploid might be concentrated by a reduction division[5]. Later, Harpstead *et al.*[6] obtained cytological evidence that the chromatin changes which took place were of the nature of point mutations, and Foster *et al.*[4] showed genetically that the mutations apparently occurred at random on different parts of the chromosomes. Colchicine may then be assumed to be a mutagenic agent. However, the true-breeding nature of these sorghum mutants could not be explained by mutation alone without supposing many identical mutations at identical loci on homologous chromosomes. This is contrary to what is known from the many studies of mutation. True-breeding mutants could, however, be formed by a reductional division subsequent to induced mutations. Likewise, appearance of sectors in F_1 flax plants homozygous for marker genes originally present in the heterozygous condition could not be explained by the mutagenic action of colchicine alone without supposing mutations at the specific marked loci which would be identical to the alleles at the corresponding loci on the homologues. Such mutations "a la carte" would be contradictory to what is known of the nature of the gene. The results reported in this paper, with the exception of those definitely due to mutation, could, therefore, have occurred solely as a result of reduction divisions within the somatic tissue of the colchicine-treated seedlings.

TABLE IV. Anomalous segregation in F_2 progeny of two colchicine-treated F_1 plants from the cross Crystal × B-5128

Branch	Rust reaction	Flower color
Plant 1	resistant: susceptible	blue: white
1	38:12	50:0
2	0:57	55:2
χ^2 for heterogeneity	64.74**	3.63
Plant 2		
1	47:2	27:22
2	10:0	1:9
3	51:0	1:50
4	7:0	0:7
5	2:0	0:2
χ^2 for heterogeneity	2.90	43.01**

**Significant beyond 1 percent point.

Summary

Branches of one F_1 flax plant treated with colchicine exhibited recessive characteristics originally present in the heterozygous condition. The plant apparently was a chimera of two sectors. Progenies of each branch (and sector) bred true, and had the normal diploid chromosome number. Anomalous segregation in the progenies of other treated F_1 plants could be explained by supposing chimeral sectors within single branches. Evidence for both recessive and dominant mutations in the progenies of treated plants was obtained. It is concluded that some of the colchicine effects noted involve somatic reduction with subsequent doubling, others mutation, and still others may be a combination of both.

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RUST PUSTULE DEVELOPMENT

On Chlorophyll-Deficient Asparagus Mutants

A. E. THOMPSON AND P. R. HEPLER*

NORMAL sporulation of rust pustules have been observed on various types of lethal, chlorophyll-deficient mutants of asparagus following urediospore inoculation with *Puccinia asparagi* DC., the causal fungus of asparagus rust.

Pustule development of both crown and stem rust has been reported not to occur on albino oat seedlings following inoculation with these pathogens. Fully susceptible reactions with abundant sporulation were produced on albino oat plants fed with various carbohydrate solutions including glucose, fructose, levulose, sucrose and maltose. Albino plants supplied only with distilled water failed to develop rust pustules[4, 5].

It appeared obvious that a basic difference exists between the reaction of chlorophyll-deficient mutants of asparagus and oats to rust infection. An experiment was therefore designed to test the extent of rust infection one might obtain on different chlorophyll-deficient mutant types of asparagus in comparison with normally pigmented plants.

Materials and Methods

Three different lethal, chlorophyll-deficient mutants were available from crosses used in experiments on methods of breeding for resistance to asparagus rust[3]. The three mutant characters were inherited as single recessive genes and genetic tests for allelism verified the non-allelic nature of the three genes (Table I). They were of two general types: One mutant was a typical albino (*a*), being devoid of visible green or yellow coloration, but purple anthocyanin coloration was rather marked. The other two were luteus types being pale-greenish yellow and indistinguishable in color. One luteus mutant was somewhat stunted in appearance and was termed dwarf luteus (*dl*) while the other luteus (*l*) mutant was normal in plant height. Lethality of the three characters is expressed in the attrition of the initial seedling spear. Only rarely has emergence of a second spear been observed among plants of the three mutants studied. However, a higher percentage of second spears has been observed in the albino than in either of the luteus mutants. The material containing the luteus and the albino genes was previously selected for a lower incidence of rust infection. The material containing the dwarf luteus gene had been previously selected for increased susceptibility to rust infection.

The experimental design was a split plot with four replications. The three genetic types made up the main plots with the alternative form of the alleles making up the subplots. Such a design allows for estimation of the interaction of the allelic forms of the three genes with regard to level of infection. Since the number of plants of each allele varied within the plots, the mean number of pustules for the contrasting alleles of the three genes were used in the analysis of variance. The use of means obviates the use of a disproportionate subclass numbers analysis.

The asparagus seeds were planted in standard seed flats. The date of emergence of the initial spears was recorded,

TABLE I. F_2 genetic data and tests for allelism of three recessive chlorophyll-deficient mutant genes of asparagus

Gene	Type of cross	Number of plants	Recessive segregates (%)
F_2 Data:			
Albino	$+/a \times +/a$	841	24.97
Luteus	$+/l \times +/l$	759	25.03
Dwarf luteus	$+/dl \times +/dl$	171	25.43
Tests for allelism:			
Albino × luteus	$+/a \times +/l$	287	0
Luteus × dwarf luteus	$+/l \times +/dl$	198	0
Dwarf luteus × albino	$+/dl \times +/a$	17	0

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thus allowing for the calculation of age of the individual spears at the time of inoculation. The spears were inoculated with urediospores 24 days after planting. Standard inoculation techniques for asparagus rust[1,3] were employed. The number of primary pustules on the main axis of the primary spear was recorded 11 days after inoculation. The 11-day period includes the incubation period plus approximately three days of sporulation.

Results and Discussion

The mean number of pustules for the various genotypes are presented in Table II. Table III presents the analysis of variance for the number of pustules. A difference in the intensity of infection was detected between the three genetic types that very nearly approached the 0.01 level of significance. Most of the variance is attributable to the higher degree of susceptibility of the material containing the dwarf luteus gene. The normally pigmented plants, which are either heterozygous or homozygous for the dominant alleles of the mutant genes, had on the average approximately 3.6 times as many pustules per plant as the chlorophyll-deficient plants.

A differential response in the level of infection of the recessive and dominant alleles of the three genes accounts for the highly significant interaction of genes × alleles. Inspection of the means in Table II will show the nature of this interaction. It is of interest to note that the two luteus type mutants responded similarly but differ from that of the albino plants, i.e., the luteus and dwarf luteus plants exhibited a greater reduction in the number of pustules as compared with their normal alleles than was shown by the albino seedlings. The albino seedlings, which must derive their total food reserve from the seed, were capable of nurturing a pathogenic fungus more successfully than the luteus seedlings, which appear to be capable of synthesizing a limited amount of carbohydrates before attrition. The possibility should not be overlooked, however, that the small amount of chlorophyll present in the luteus types might not function in photosynthesis. Since the lines were not isogenic, differences in the residual genotype upon which the mutants operate cannot be ruled out as a primary factor in the differential response of the allelic forms of the genes. Such a differential response is difficult to rationalize if carbohydrate reserve in the plant is the sole determining factor for normal rust pustule development in chlorophyll-deficient plants as Tegenkamp and Finkner's[4,5] data suggest.

Increased age of the spear of relatively mature asparagus plants is known to reduce the level of rust infection[1,3]. With increased age of a chlorophyll-deficient asparagus spear one might reasonably expect a depletion of the carbohydrate level of the plant. The age of the initial spear of the tested plants was recorded in days from the time of spear emergence to the time of inoculation. Table IV presents a summary of the number of plants and the mean number of rust pustules on spears of differing ages at the time of inoculation for the contrasting type of allele. The data for the three genes were pooled as only minor variations in infection attributable to spear age were observed between the three genetical types. The mean spear age at time of inoculation was very nearly the same for plants of the dominant and recessive classification. The normal plants on the average were only approximately one-half day older than the mutants.

The effect of seedling spearage on rust infection in normally pigmented plants is clearly demonstrated in Table IV. The general decrease in susceptibility associated with increasing age of spears has been attributed largely to the morphological differentiation of tissues and thickening of secondary cell walls within the spear. However, chemical changes within the spear associated with increasing age may well be as important as morphological differentiation of tissues and cell walls in modifying susceptibility of the spear. The same effect is present in the chlorophyll-deficient plants but the decrease in the level of infection appears accentuated with increasing age. This latter effect undoubtedly is due in part to the depletion of the food reserves. Even though age of the spear has a marked effect on the level of infection, it was possible to infect chlorophyll-deficient plants at all ages included in the experiment. The data indicate that the chlorophyll-deficient plants reach their maximum level of infection ap-

TABLE II. Mean number of rust pustules per plant on allelic forms of three lethal, chlorophyll-deficient mutant genes of asparagus

Type of gene	Type of allele — Dominant	Recessive	Mean
Albino	8.18	5.36	6.77
Luteus	7.49	1.50	4.50
Dwarf luteus	20.71	3.20	11.95
Mean	12.13	3.36	7.74

TABLE III. Analysis of variance of number of rust pustules per plant on normal and chlorophyll-deficient asparagus seedlings

Source of variation	Degrees of freedom	Mean square
Genes	2	116.8*
Replications	1	15.5
Error (a)	6	12.6
Alleles	1	461.6**
Genes × alleles	2	119.6**
Error (b)	9	8.0
Total	21	

* Exceeds 0.05 level of significance.
** Exceeds 0.01 level of significance.

proximately two days earlier than the normal plants. This may suggest that chlorophyll-deficient plants are proportionately more highly susceptible than normal plants at a younger age.

The data presented clearly indicate that chlorophyll-deficient plants of asparagus are basically different from chlorophyll-deficient plants of oats in their reaction to rust infection. Certain points of similarity and difference between the two species may be made. Both species are monocotyledons, but the represented families are rather divergent from a structural and evolutionary standpoint. The oat plant is an annual and asparagus is a herbaceous perennial; that is, the asparagus spear is annual in its growth habit. The average seed weight of oats and asparagus is approximately the same. An oat seed will average from 20 to 22 mgms. in weight, of which approximately 25 percent is hull. The average seed weight of the asparagus crosses used in this experiment was 22.7 mgms. thus indicating that asparagus and oat seeds are of approximately the same total weight. However, the proportion of stored carbohydrate to seed coat would be much greater in asparagus seeds. The very thin seed coat of asparagus seeds would not constitute more than one or two percent of the total seed weight. Basic differences in chemical content of asparagus and oat seeds are perhaps significant. The chief reserve carbodydrate of asparagus is in the form of hemicelluloses[2] while that of oats is chiefly starch. Asparagus seeds contain from 12 to 13 percent fat,[2] which is approximately twice the amount found in oat seeds.

The relevance of the above listed factors to the observed difference in rust infection between chlorophyll-deficient plants of the two species has not been determined. Qualitative and quantitative differences in carbohydrates also appear to be of importance. Tegenkamp and Finkner[5] concluded that a greater percentage of inoculated albino oat plants developed pustules when maltose or glucose was supplied than when fructose or levulose was supplied. Concentration of solutions also affected rust pustule development. Other factors such as vitamin or hormone content may be equal to or of more significance than carbohydrate level in regulating such a response.

Summary

The nature and extent of rust infection on three non-allelic, recessive, lethal, chlorophyll-deficient mutants of asparagus were studied. Normal pustule development and sporulation were observed on all mutants. In all instances the level of infection of the mutant plants was lower than the normal plants, but the allelic forms of the three genes responded differentially to infection. The intensity of infection was significantly greater on albino (*a*) plants than on the luteus (*l*) and dwarf luteus (*dl*) mutants. The differential response was interpreted as indicating that carbohydrate level is not the sole determinant of infection level of chlorophyll-deficient asparagus plants. Increasing age of spears had more effect in reducing infection on mutant than on normal plants, but infection was obtained on mutant plants at all ages tested in the experiment. These results differ basically from those that have been reported to occur on albino oat plants where normal rust pustules develop only if an external carbohydrate source is supplied to the plant.

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TABLE IV. Effect of spear age on rust infection of asparagus plants classified on the basis of allelic form of chlorophyll-deficient mutant genes

Spear age at inoculation (days)	Type of allele: Dominant — Number of plants	Dominant — Mean number of pustules	Recessive — Number of plants	Recessive — Mean number of pustules
1	3	0.33	2	2.50
3	7	1.00	10	5.50
5	25	9.16	18	6.17
7	94	10.63	31	5.42
9	194	8.35	60	2.95
11	244	7.50	68	1.88
Mean spear age at inoculation		8.74		8.11

A REANALYSIS OF THE PROBLEM OF THE MALE TORTOISESHELL CAT

LUCIAN M. SPRAGUE AND CLYDE STORMONT*

NUMEROUS papers have been devoted to the subject of the nature and origin of the tortoiseshell male cat. Few, if any, have been amenable to direct experimental verification. The present report is concerned with still another explanation which we feel can be tested. Considering the rarity of these animals our purpose is also in part an appeal for complete litters containing a tortoise male or males.

Following the discovery of the phenomenon of erythrocyte mosaicism in cattle twins by Owen[13], there has been increasing interest in the consequences of vascular anastomoses, a topic recently reviewed by Stormont[16]. In a study of the literature on the inheritance of coat colors in cats, it occurred to us that the puzzling features regarding the origin of the tortoiseshell male might be explained as a consequence of chorionic vascular anastomosis between litter mates of contrasting color types.

The vast majority of tortoiseshell cats are female. The commonly accepted explanation for the inheritance of tortoiseshell color is as follows: a pair of sex-linked alleles designated by us as b^1 and b^2 are responsible for black, orange and tortoiseshell coat colors. The allele b^1 when hemizygous (b^1/Y)† or homozygous (b^1/b^1) produces black. The allele b^2 when hemizygous (b^2/Y) or homozygous (b^2/b^2) produces orange. Heterozygotes (b^1/b^2) are tortoiseshell females. However, a few tortoiseshell males occur. Most but not all of these tortoiseshell males have proven to be sterile. The genotypes of the fertile males have never been adequately tested. Three fertile tortoiseshell males, recorded in the literature (Doncaster[5], Bamber[2]), apparently transmitted only yellow to their offspring.

The color phenotype of any given animal, regardless of sex, is subject to the modifying action of genes at several independent loci. The expression of genotypes conditioning black is modified by alleles situated at the Agouti locus as well as by those genes effecting spotting with white, color intenstiy, and extension and restriction of black. Whiting[17], in reviewing this problem concluded in effect that phenotypic appearance may not reflect a unilateral correspondence with genotype in a few cases. He was of the opinion that a number of tortoiseshell females have been misclassified as black. The effect of this misclassification is that female offspring which appear to be of exceptional color may be produced (Table I).

Table I shows the six possible kinds of matings involving black, orange and tortoiseshell cats and the offspring of these matings as compiled by Doncaster in 1913[5], and Wright in 1918[20]. Table I shows that among the male offspring the exceptional types are limited to three tortoiseshell cats in a total of 294. Among the female progeny listed in Table I, 22 exceptions occur in a total of 263. It is believed by the present authors that the majority of the exceptions among the female offspring could be the result of phenotypic misclassification of the parents. The three tortoiseshell females resulting from a cross of an orange male and an orange female listed in Table I are almost certainly of this type since they are full sibs, but not litter mates.

The occurrence of tortoiseshell males which are usually, but not always, ster-

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†Y symbolizes hemizygosity throughout the discussion.

ile has provoked the following explanations; failure of sex linkage[5], non-disjunction[11], sex reversal[6], and crossing-over in homologous regions of the X and Y chromosomes[10]. Of these explanations only Doncaster's[6] will be treated here.

Discussion

Doncaster[6] proposed that tortoiseshell males were actually *genetic females* which had undergone partial or total sex inversion as a consequence of chorionic vascular anastomosis with a male litter mate. This postulate was put forward as an analogy with the free-martin condition found in cattle, but extends the degree of effects of vascular anastomoses in that it proposes complete (male fertile) or partially complete (male sterile) sex reversal in the *genetically female* member of unlike sexed pairs. Doncaster's hypothesis has been abandoned by subsequent workers on the basis of no evidence to suggest anatomical gradations between females and males due to sex transformation[8], and the absence of anatomical vestiges of female reproductive organs in tortoiseshell males, demonstrated by Wislocki and Hamlett in 1934[18]. Doncaster's hypothesis is important, however, in suggesting vascular anastomoses as a possible mechanism of the origin of tortoiseshell males.

Vascular anastomoses can be diagnosed by two methods; (1) a demonstration of communal circulatory systems between the fused chorions of a multiple birth, or (2) a serological demonstration of populations of erythrocytes of different zygotic origins (erythrocyte mosaicism).

Vascular anastomoses and sterility resulting from communal circulatory systems are clearly disjunct. Under conditions of heterosexual vascular anastomoses, four possibilities exist with respect to sterility. Namely, no sterility will result, the female will be sterile and the male fertile, the male will be sterile and the female fertile, or neither member will be fertile.

Representative of type one are marmosets. Wislocki in 1939[14] reported that in marmosets where twinning associated with vascular anastomosis is highly frequent no evidence exists to suggest that either member of a heterosexual pair of marmosets is sterile as a result of vascular anastomoses. In man, Dunsford *et al.*[7] have reported an example of erythrocyte mosaicism in a female, Mrs. M. K. The co-twin, a male, died before reaching reproductive age. Mrs. M. K., however, had one offspring. This example may be either of type one or type three. (See also Perez *et al.*[14] for a description of the occurrence in man of vascular anastomoses in the placentas of dizygotic individuals.)

Four species of Ungulates exemplify type two, cattle,[12] goats,[9] sheep,[16] and some swine.[8]

It may be that the male member of a heterosexual pair of vascular anastomosed cats exemplifies either type three or type four. A choice between these two alternatives does not, however, affect the consequences of our interpretation.

It is suggested here that the rare tortoiseshell male is the result of chorionic vascular anastomosis between individuals of contrast-

TABLE II. A summary of the ways in which tortoiseshell males could occur by vascular anastomoses

Parental Genotypes		Genotype of Offspring		Tortoiseshell Males Actually Observed		
Males	Females	Males	Females	*	**	***
$\underline{b}^2/Y$ × $\underline{b}^1/\underline{b}^1$		$\underline{b}^1/Y \leftrightarrow \underline{b}^1/\underline{b}^2$		1	1	
$\underline{b}^1/Y$ × $\underline{b}^1/\underline{b}^1$		$\underline{b}^1/Y$	$\underline{b}^1/\underline{b}^1$	0	0	
$\underline{b}^1/Y$ × $\underline{b}^2/\underline{b}^2$		$\underline{b}^2/Y \leftrightarrow \underline{b}^1/\underline{b}^2$		0	0	2
$\underline{b}^2/Y$ × $\underline{b}^2/\underline{b}^2$		$\underline{b}^2/Y$	$\underline{b}^2/\underline{b}^2$	0	0	
$\underline{b}^1/Y$ × $\underline{b}^1/\underline{b}^2$		$\underline{b}^1/Y$ ↕ $\underline{b}^2/Y \leftrightarrow \underline{b}^1/\underline{b}^2$	$\underline{b}^1/\underline{b}^1$ ✕	1	2	4
$\underline{b}^2/Y$ × $\underline{b}^1/\underline{b}^2$		$\underline{b}^1/Y \leftrightarrow \underline{b}^2/\underline{b}^2$ ↕ $\underline{b}^2/Y \leftrightarrow \underline{b}^1/\underline{b}^2$	↘	1	3	

$\leftrightarrow$ indicates presumed ways in which tortoiseshell males may be produced as a consequence of vascular anastomoses.

* From Doncaster[6], Wright[20].

** From Komai[10].

*** From Komai[10], sires of unknown or uncertain color.

TABLE I. Six possible kinds of matings involving black, orange and tortoiseshell cats*

Type of Mating	Offspring: Males — Black b^1/Y	Males — Tortoise	Males — Orange b^2/Y	Females — Black b^1/b^1	Females — Tortoise b^1/b^2	Females — Orange b^2/b^2
1. Orange b^2/Y × Black b^1/b^1	51	1†	0	11†	51	0
2. Black b^1/Y × Orange b^2/b^2	0	0	20	0	16	0
3. Orange b^2/Y × Orange b^2/b^2	0	0	48	0	3†	40
4. Black b^1/Y × Tortoiseshell b^1/b^2	32	1†	37	14	22	0
5. Orange b^2/Y × Tortoiseshell b^1/b^2	45	1†	58	6†	45	53
6. Black b^1/Y × Black b^1/b^1	No exceptional types on record					

*Compiled from Doncaster,[5] Wright.[20]

†Exceptional offspring. See text.

ing genotypes resulting in a color pattern conditioned by cells of genetically different origins. Further, if the anastomosed pair is of unlike sex, sterility results in the *male*.

The consequences of this postulate are summarized, insofar as the males are concerned, in Table II. We have adopted the symbol ⟷ to indicate the occurrence of vascular anastomoses between a pair of litter mates. Although no tortoiseshell male animals have been recorded from matings between black males (b^1/Y) and orange females (b^2b^2) in Table II, we would expect that tortoiseshell males could occur in such a mating. The data of Komai[10] lists two tortoise males in litters from orange females, the coat color of the sires is not known but might be assumed to be black.

Observations essential to this explanation based on anastomoses were reported by Wislocki and Hamlett.[18] They describe finding the occurrence of synchorial litter mates in cats as a result of a routine examination of a series of 19 pregnant feline uteri. In one uterus containing six fetuses, there was extensive union of the fetal membranes of a pair of fetuses of unlike sex. The reproductive tracts of the pair revealed no apparent abnormalities. In this same study, Wislocki and Hamlett also examined an apparently normal male tortoiseshell cat. The reproductive structures appeared to be completely male and of normal size and proportions. Careful dissection revealed no representation of female parts in the abdomen or pelvis. The only abnormality observed was the absence of spermatogenesis in the testes of this male. Wislocki's and Hamlett's findings do not support a hypothesis based on sex inversion, but do not exclude the possibility that a sterile male tortoiseshell cat is the result of fusion of the chorionic vessels of an unlike sexed pair.

One might well ask at this point, "What fraction of the males which are encountered in the general population of cats might be expected to be sterile, on the basis of placental fusion, regardless of their color pattern?" From the various literature sources available 237 pregnant uteri containing 985 fetuses have been examined with specific emphasis on determining the frequency of synchorial litter mates. Of these fetuses, two showed "extensive union of the fetal membranes." If a value of 1 pair in 1×10^3 fetuses is assigned as a reasonable frequency for the occurrence of synchorial litter mates in cats, then approximately 1 pair in 2×10^3 fetuses will involve an unlike sexed pair, or 1 male in 1×10^3 male fetuses would be involved in such synchorial pairs. Consequently, approximately 1 male per 1×10^3 would be expected to be sterile as a result of vascular anastomosis with a female litter mate. All of these males would by no means be tortoiseshell since the appropriate matings with respect to color are made in general by chance alone.[1,2] With respect to tortoiseshell males, R. D. Owen has pointed out to us that black and yellow embryos of like sex occur as a function of the frequency of tortoiseshell females, ie. 2 (pq) where p = allele frequency of b^1 and q = b^2. The frequency of fertile tortoiseshell males would then be equal to .5 (pq)k where k is the frequency of anastomoses. (ie. k/4 = proportion of male-male anastomoses.)

If our hypothesis is correct we would expect that tortoiseshell males would be tolerant to homografts of skin from one of their litter mates as suggested by the findings of Anderson *et al.*[1] in their studies of cattle twins; providing of course that each of the anastomosed members are among the living members of the litter.

Summary

It is postulated from the available data that the male tortoiseshell cat may be a mosaic of cells of genetically different origins resulting from the occurrence of vascular anastomoses between synchorial litter mates of contrasting genotypes.

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THE FUTURE OF ARID LANDS

Representatives from 28 countries met to consider the present state of research and development on arid zone problems and to formulate recommendations for new lines of research. Leaders from 18 of these countries gave papers at the symposium in Albuquerque, New Mexico, and joined in the Conference at Socorro, New Mexico, in formulating a series of recommendations. The symposium papers and the 31 Conference recommendations are reproduced in this volume.*

The symposium sessions were developed around a few basic questions. The representation and treatment of the subjects were highly interdisciplinary and led to some important conclusions. The breadth and scope are indicated by the groupings of the Conference recommendations: Anthropology, Archaeology and Geography; Meteorology and Climatology; Hydrology, Geology and Soils; Biology, Ecology and Conservation; Organization, Communication, and Interdisciplinary Programs. Workers in all these fields, as well as administrators of government and private programs, will find the contents of this volume both stimulating for ideas and an invaluable source of information.

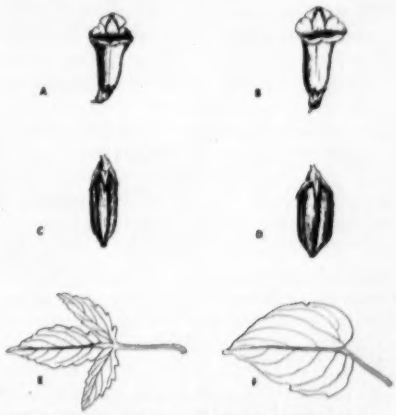
*The Future of Arid Lands, edited by Gilbert F. White, Department of Geography, The University of Chicago. 464 pages, 49 illustrations, index, clothbound. $6.75. 1956. A symposium volume of the American Association for the Advancement of Science. AAAS Members' prepaid order price, $5.75.

PRODUCTION OF FERTILE AUTOTETRAPLOIDS IN SESAME

And Their Breeding Behavior

R. N. SRIVASTAVA*

THE present investigation was undertaken to develop tetraploids of a pure variety of Sesame, *Sesamum orientale* L. Seeds of a pure variety of *Sesame orientale* L. were soaked in water for 12 hours and treated with 0.04, 0.06, 0.08 and 0.10 percent aqueous solution of colchicine for varying lengths of time. After treatment the seeds were washed thoroughly with water and germinated in earthen pots in ordinary soil. Detailed results are given in Table I. Few treated seedlings developed into mature plants. Out of these, three were tetraploids. Twenty-five seeds treated with 0.04 and 0.06 percent for two and six hour periods produced one tetraploid each, and 25 seeds treated with 0.10 percent for four hours also produced one tetraploid. Table I gives the number of plants in the different treatments that grew to maturity and the proportions of polyploids in each treatment.



MORPHOLOGICAL CHARACTERISTICS

Figure 20

A and *B*—Flower of diploid and of tetraploid. *C* and *D*—Capsule of diploid and of tetraploid. *E* and *F*—Leaf of diploid and of tetraploid.

General Characteristics of Colchicine-treated Plants

C_1 Generation

In early seedling stages, the cotyledons of the tetraploids were usually larger, rounder, darker, and thicker than those of the corresponding diploids. The tetraploid seedlings grew slower but at maturity were about 1½ times the size of the diploid plants. Stems of tetraploids were thicker and more prominently ribbed. Their leaves were larger, broader, thicker and greener. The most striking morphological change was leaf shape in the tetraploids. Except for one or two basal leaves, all the leaves of the diploid plants of sesame were tripartite, serrate, and yellowish green. Leaves of tetraploids were simple, wavy, and very dark green.

Size of stomata increased in tetraploids; frequency decreased. The average size of stomata in the tetraploid was 42×30 sq.μ and in the diploid it was 28×20 sq.μ. Flowers of tetraploids were larger than those of diploids, and the ovaries of tetraploids were broader. Flowering date was a week or so later than that of the diploids.

The diameter of the pollen grains in the tetraploid was 46.3μ, and the diameter of the diploid pollen grains was 33.4μ. Difference in fertility between tetraploid and diploid was not significant. The rate of maturity in tetraploid fruits was slower than in diploid fruits, but the tetraploid fruit was longer and broader and more hairy, whereas the diploid fruits were smooth.

C_2 Generation

Seeds from two plants and from individual branches of the third tetraploid (which had one triploid branch) were collected and field grown in 1953

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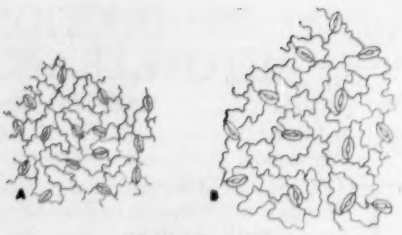
together with controls. Seeds collected from tetraploid plants obtained from seeds treated with 0.04 percent colchicine solution for two hours did not germinate at all. Seventy-two seeds were obtained from the autotetraploid plant which was produced after treatment with 0.06 percent colchicine solution for six hours. Only 49 plants survived, of which 47 were autotetraploids and two were mixoploid. One hundred sixty-seven seeds were obtained from the tetraploid plant after treatment with 0.1 percent colchicine solution for four hours which gave rise to 129 autotetraploids and three mixoploid plants. Seedlings which resulted were studied morphologically and chromosome numbers of the individual plants were determined. Reciprocal crosses of diploids and tetraploids were made. The nature of progenies from the different plants is given in Table II, and data on morphological characters in Table III.

Table II shows that in two cases seeds from different branches produced plants with different chromosome numbers.

Table III brings out some of the morphological changes induced in the sesame plants as a result of tetraploidy. Cell size in the tetraploid is increased as shown by the stoma guard cells and the pollen. The dimention of the guard cell



STOMATA SIZE

Figure 21

A—Stomata on the lower epidermis of leaves of the diploid. *B*—Stomata on the lower epidermis of leaves of the tetraploid.

of a tetraploid is 42.18 × 22.6 μ and that of the diploid is 28.18 × 21.97 μ. The diameter of tetraploid pollen is 45.08±1.53 μ and that of diploid is 39.61 ± 038 μ. The area of the leaf lamina and the flowers are also increased, (Figure 20*B* and *F*). The length and breadth of a tetraploid leaflet is 19.0 ± 0.28 cm and 8.95 ± 0.75 cm respectively, and that of a diploid is 14.36 ± 0.80 cm and 6.26 ± 0.17 cm respectively. The maximum length of the tetraploid flower is 4.19 ± 0.15 cm and that of the diploid is 3.3 ± 0.10 cm. The increased size of these plant cells is shown in Figure 21*B*. The capsule size is also larger in the tetraploid than in the diploid.

TABLE I. Result of colchicine treatment of sesame

Concentration of colchicine	0.04					0.06					0.08					0.10					Control		
Duration of treatment	No. of seeds treated	No. of plants obtained	No. of polyploids	Percent survival	Percent polyploids	No. of seeds treated	No. of plants obtained	No. of polyploids	Percent survival	Percent polyploids	No. of seeds treated	No. of plants obtained	No. of polyploids	Percent survival	Percent polyploids	No. of seeds treated	No. of plants obtained	No. of polyploids	Percent survival	Percent polyploids	No. of seeds treated	No. of plants obtained	Percent survival
2 hours	25	19	1	76	4	25	17	0	68	0	25	15	0	60	0	25	10	0	40	0	25	20	80
4 hours	25	17	0	68	0	25	15	0	60	0	25	14	0	56	0	25	9	1	36	4	25	19	76
6 hours	25	15	0	52	0	25	13	1	52	4	25	11	0	44	0	25	6	0	24	0	25	20	80
12 hours	25	7	0	28	0	25	10	0	40	0	25	4	0	16	0	25	4	0	16	0	25	17	68

TABLE II. Progenies of colchicine treated plants in the C_2 generation (1953)

Plants in 1952	No. of seeds sown	No. of plants obtained	No. of diploids $2n = 26$	No. of polyploids $2n = 52$	Remarks
1/2/.04	49	0	0	0	No germination took place
1/6/.06	72	49	0	49	Two plants were aneuploids
1/4/.1	167	132	0	130	One octoploid

The length of the capsule of the tetraploid is 2.95 ± 0.03 cm and that of the diploid is 2.46 ± 0.04 cm, (Figure 20 *C* and *D*, and Table III). The height of the tetraploid sesame has significantly increased to 82.0 ± 7.6 cm in comparison to the diploid which was 49.6 ± 2.4 cm. Difference in sterility between tetraploid and diploid was not significant. The sterility in the former varies from 20 to 25 percent while in the latter it varies from five to 25 percent.

The range of variability of some of the morphological characters studied is greater in tetraploids than in diploids. It appears from the data that there is more variability in height and slight variability in sterility of tetraploids when compared to diploids.

Cytology

Preliminary detection of polyploidy in the treated plants was based mainly on the increased size of stoma guard cells and pollen. As soon as buds were available, the chromosome number was determined from P.M.C. smears in aceto-carmine according to Bellings[1] method, at which time the tetraploid nature was confirmed.

The somatic chromosome number of *Sesamum orientale* L. has been determined by Richharia[7] and Ramanujam[6] as 26. Polyploid species of sesamum are unknown, but Ramanujam[6], however, reported the somatic number of artificially produced polyploids of *Sesamum orientale* as 52.

In the present investigation in the root-tips of the diploid, 26 chromosomes were seen while in the tetraploids 52 chromosomes were clearly counted.

MEIOSIS IN THE TETRAPLOID

Figure 22

A—Metaphase II in the tetraploid with 26 chromosomes at each pole. *B*—Anaphase I in the tetraploid. *C*—Anaphase II in the tetraploid.

In pollen meiosis in the tetraploids varying numbers of quadrivalents and bivalents were formed at diakinesis and metaphase I, the total number of chromosomes in each cell being 52, (Figure 22*A-C*). In two plants only, with 95 percent pollen sterility, the chromosomes were distributed unequally to the two poles. The frequency of chromosomes to the two poles at anaphase I were observed to be 26 + 26, 24 + 28 and 22 + 30.

Crossing between diploid and autotetraploid varieties

The colchicine produced autotetraploid varieties of *Sesamum orientale* together with three diploid varieties, namely the original diploid which was used for inducing the polyploidy, Ml, and Bhl were the materials used in crossing and in the reciprocal crosses.

TABLE III. Morphological characteristics

Parent characters	Tetraploids: Mean ± standard error	Tetraploids: Standard deviation	Diploids: Mean ± standard error	Diploids: Standard deviation	Difference between means	S.E. of difference	M.D. S.E. of difference
1. Pollen diameter	45.08 ± 1.53	5.44	39.61 ± .038	1.48	5.47	1.51	3.5*
2. Pollen sterility	20 to 25%	———	5 to 25%	———	———	———	———
3. Height of plant	82.0 cm. ± 7.6	24.13	49.6 cm. ± 2.4 cm.	7.63	32.40 cm.	7.9	4.1*
4. Length of leaflets	19.0 cm. ± .28	.689	14.36 cm. ± .8	3.188	4.64 cm.	0.84	5.5*
5. Breadth of leaf	8.95 cm. ± .75	1.85	6.26 cm. ± .17	.43	2.29 cm.	0.76	3.0*
6. Length of the guard cell of stomata	42.18 ± 1.98	7.25	28.18 ± 1.1	3.65	14.0	2.26	6.0*
7. Breadth of the guard cell of stomata	22.6 ± .87	2.85	21.97 ± .58	1.85	0.63	1.04	.6 Insignificant
8. Maximum length of flower	4.19 cm. ± .15	0.37	3.3 cm. ± .10	0.26	0.89 cm.	0.18	4.9*
9. Length of capsule	2.95 cm. ± .03	0.077	2.46 cm. ± .04	.109	0.49 cm.	0.05	9.8*
10. Breadth of capsule	1.11 cm. ± .06	0.148	.76 cm. ± .036	.089	0.35 cm.	0.06	5.8*
11. No. of capsules per plant	114 to 61	———	167 to 67	———			

*Denotes significant.

(a) 2x (♀) × 4x (♂). Among 50 emasculated diploid flowers of each variety pollinated with pollens from tetraploid plants, 12 strikingly poor capsules were developed. Close observations revealed that at an early stage when these capsules were still immature, they contained large and well developed seeds. When mature these capsules were in a more or less seedless state except for some shrivelled, small and empty seeds, with no contents whatsoever.

(b) 4x (♀) × 2x (♂). In the case where the tetraploids were used as female parents and were pollinated with the pollens of M1 and Bh1 diploids (not of the same diploid variety) the development of the capsule was poor and only a few small empty seeds out of 50 emasculated flowers were obtained. When the tetraploid flowers were crossed with their own diploid progenitors the result was different. Out of 50 emasculated flowers used in crossing, 30 capsules having 51 normal looking but small seeds developed.

Discussion

The tetraploid race of *Sesamum orientale*, which has been developed, differs from the diploid as much as have previously reported tetraploids[1,2]. It has larger leaves, thicker stems, larger capsules, larger pollen grains, larger stomata and larger flowers. The leaf shape of diploids is tripartite and that of the tetraploid is simple. This is a striking change in leaf shape that may be considered of evolutionary significance.

The main difficulty in the use of autotetraploids in a plant breeding program is due to their sterility and poor seed set. In sesame autotetraploids, the pollen sterility varies from 20 to 25 percent, and in diploid from five to 25 percent. The number of capsules in autotetraploids vary from 144 to 61 and in diploids from 167 to 67. Similar results of high fertility were reported for a tetraploid Buckwheat and *Sesamum indicum* produced by colchicine treatment. This high fertility is due to some mechanism whereby quadrivalents and other polyvalent chromosome associations are replaced by bivalents which has been confirmed by cytological studies.

As stated above, the hybridization has been successful only in the direction of the cross *4x* (♀) × *2x* (♂) in *Sesamum orientale* where *2x* (♂) was its own progenitor. In the other direction of the cross and also where *2x* (♂) of different varieties were used, empty and non-viable seeds have always been obtained. However, in similar crosses between diploids and tetraploids in the closely related species, *Brassica chinensis*, *Brassica pekinensis* and *Brassica rapa*, empty and non-viable seeds were usually set in both directions of the cross (Nishiyama *et al.*)[5]. From these results it appears that the isolation of tetraploid varieties of *Sesamum orientale* from diploid varieties other than its own progenitor is not necessary as out-crossing is not possible.

Sesame being a self fertilized crop, fertility can be improved by selection. Another way of improving the fertility of these autotetraploids would be to cross them with similar polyploids evolved from other diploid varieties and then, by selection for a few generations as suggested by Kuckuck and Levan[3].

Summary

Soaked seeds of Sesame were subjected to varying treatments with aqueous colchicine solution. The C_1 and C_2 generations were studied. Treatment of seeds with 0.06 percent for six hours and 0.10 percent for four hours gave the best results for the induction of polyploidy. In the C_1 generation one plant was mixoploid and the others were autotetraploids.

The detailed study of morphological characters of the C_2 generation showed that the tetraploid sesame possesed larger and simple leaves, bigger flowers, larger capsules and bolder seeds and longer plants than diploids. The pollen grains and stomata guard cells of tetraploids were bigger than those of diploids. The sterility of the tetraploids was 20 to 25 percent and that of diploids five to 25 percent.

The chromosome numbers of tetraploids and diploids were 52 and 26 respectively. Meiosis in the tetraploids was fairly regular. Tetraploid plants gave rise to tetraploid progenies.

The diploids could not be crossed with polyploid males but in some cases reciprocal crosses with their progenitor were successful.

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CROSSBRED DWARFS IN BEEF CATTLE

MARTIN J. BURRIS AND B. M. PRIODE*

TYPICAL DWARFS

Figure 23

Dwarf calves produced at the Beef Cattle Research Station, Front Royal, Virginia. Approximate age of calves was five months. *A*—Hereford dwarf; *B*—(foreground) Angus dwarf; *C* and *D*—Hereford × Angus dwarf.

DURING recent years many research workers have reported a type of dwarfism in beef cattle whose occurrence has apparently become more frequent. This type, described as a thick short-legged dwarf with bulging forehead, undershot lower jaw, and protruding tongue[3], has occurred so often in certain breeds and certain herds as to cause much concern and considerable economic loss. It has popularly been referred to as the "snorter-type" dwarf. The Hereford dwarf reported by Johnson *et al.*[2] was of this general type. These workers indicated that this dwarf type was conditioned by a single autosomal recessive gene in the homozygous state. Chambers *et al.*[1] have reported the occurrence of 11 dwarf calves in 37 calves from Comprest × Comprest matings. "Comprest" is an expression popularly used to describe Hereford animals which are extremely short and blocky, a condition believed caused by the "Comprest" gene when present in the heterozygous state. Comprest animals are intermediate to conventional and dwarf animals in many characteristics including height, conformation and facial characteristics. Both Hereford and Angus dwarfs with the general physical characteristics of the snorter-type dwarf have been produced at the Beef Cattle Research Station at Front

*Virginia Agricultural Experiment Station and U.S.D.A., Beef Cattle Research Station, Front Royal, Virginia, respectively.

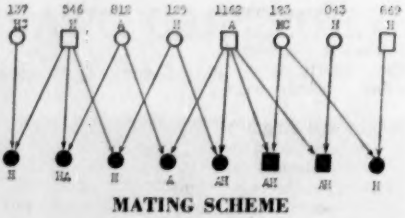
Royal, Virginia (see Table I, calves born in 1954, and Figure 23).

That these dwarfs may also be caused by the action of a recessive genetic gene in the homozygous state in each breed has been indicated by the parental phenotypes, the small proportion of dwarfs in comparison to non-dwarf calves, and the occurrence of dwarfs in only certain breeding groups. Since the Hereford dwarfs and the Angus dwarfs are similar in appearance, a question which naturally arises is, "what is the relationship of the genes causing dwarfism in the two breeds?"

Mating Results

In 1955 and 1956 calves were produced from matings of animals known or suspected of being dwarf carriers. Group A included seven daughters of a Comprest type Hereford bull and four of the cows were themselves of the Comprest type. One of these Comprest cows had previously produced a dwarf calf when bred to a Hereford bull of normal phenotype. The dams of cows in Group A, however, had produced no dwarfs when they and related cows were mated to a bull which had sired dwarfs. Cows in Group B were eight daughters of a known dwarf-producing bull. In the spring of 1955 these two groups of cows produced calves after being bred to an Angus bull which had previously sired a purebred dwarf Angus calf. In the spring of 1956 four cows from Group A, four cows from Group B, and one Angus cow produced calves when bred to a Hereford bull whose sire had produced dwarfs.

The progeny of (the matings of the) Group A cows and the Angus bull included one crossbred dwarf from a Comprest cow and six non-dwarf crossbred calves. The cow which produced the crossbred dwarf was the same individual which had previously produced a Hereford dwarf when mated to a conventional-type Hereford bull. The progeny of the mating of the Angus bull to daughters of a dwarf carrier Hereford bull (Group B) included three crossbred dwarfs and five crossbred non-dwarf



MATING SCHEME

Figure 24

Illustrated graphically are some of the matings that produced dwarf offspring, (H is Hereford, A is Angus, C is Comprest).

calves. The calves produced in 1956 include three dwarfs, one from a Comprest cow in Group A, one from a cow in Group B, and one from the Angus cow. Both the last two cows had previously produced dwarfs, but the Comprest cow in Group A had not previously produced a dwarf calf. Six non-dwarf calves were produced by the cows in Group A and Group B from this mating. The matings which produced dwarf calves are shown diagrammatically in Figure 24. The total number of dwarf and non-dwarf calves produced from these matings are shown in Table I.

The crossbred dwarfs produced were phenotypically similar to the dwarfs produced by Hereford × Hereford matings and Angus × Angus matings, (see Figure 23). However, the crossbred dwarfs showed some indication of heterosis if this small sample may be an indication. The crossbred dwarfs were more vigorous, less susceptible to scours and dietary disorders, and larger framed when compared with either Hereford or Angus dwarfs. Weights of individual dwarf calves adjusted to 120 days of age were: a female Angus × Hereford, 235 pounds; a male Angus × Hereford, 199 pounds; an Angus female, 211 pounds; a Hereford female, 165 pounds; inbred Hereford dwarfs (sire × daughter mating) female, 173 pounds; female 123 pounds; and male, 163 pounds. The average weights of all normal calves produced at the Beef Cattle Research Station in the 1955 season adjusted to 120 days of age were: Hereford female, 254 pounds; Hereford male, 278 pounds; Angus female, 255 pounds; and Angus male, 276 pounds.

TABLE I. Dwarf and normal calves produced from various mating groups

Sire	Year Born	Type of Dams	Sire of Dams	Normal Calves	Dwarf Calves
Angus 1162	1954	Angus		15	1
	1955	Comprest Hereford	Comprest Hereford	5	1
	1955	Conventional Hereford	Comprest Hereford	3	0
	1955	Conventional Hereford	Carrier Hereford	5	3
Hereford 869	1954	Comprest Hereford	Comprest Hereford	0	1
	1954	Hereford		3	0
Hereford 546	1956	Angus		0	1
	1956	Comprest Hereford	Comprest Hereford	1	1
	1956	Conventional Hereford	Comprest Hereford	2	0
	1956	Conventional Hereford	Carrier Hereford	1	1

Summary and Conclusions

Dwarfs phenotypically similar to dwarfs of the "snorter-type" previously described by other workers were produced by Angus × Angus, Hereford × Hereford, Hereford × Angus, and Angus × Hereford matings when all parents used were "apparently" phenotypically normal and of conventional type. Phenotypically indistinguishable dwarf calves were produced when Comprest Hereford cows were mated to Hereford bulls and to an Angus bull. In some instances more than one dwarf was produced by one individual cow in successive seasons.

The occurrence of crossbred dwarfs from these matings indicates that the factors causing dwarfism in the two breeds act in a similar manner whether the parents in a given case are of the same or different breeds. This observation lends considerable support to the hypothesis that the recessive genes causing dwarfism in the two breeds are either the same gene or alleles with very similar effects.

The apparent increased growth rate and greater vigor of the crossbred dwarfs as compared to purebred dwarfs indicates that the expression of the homozygous recessive dwarf genotype may be influenced by the genetic background of the animal.

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RESOLUTION OF THE CONGRESS OF HUMAN GENETICS

Resolutions adopted by the First International Congress of Human Genetics, on the subject of radiation damage in response to a request by Dr. Eve of WHO for an expression of the viewpoint of congress members on this subject.

The damage produced by ionizing radiation on the hereditary material is real and should be taken seriously into consideration in both the peaceful and military uses of nuclear energy as well as in all medical, commercial and industrial practices in which X-rays or other ionizing radiation is emitted. It is recommended that the investigation of the amount and type of damage and of related genetic questions, be greatly extended and intensified with a view to safeguarding the wellbeing of future generations.

INHERITED MUSCLE ABNORMALITY IN THE DOMESTIC FOWL

V. S. Asmundson and L. M. Julian*

MANY abnormalities, both lethal and nonlethal, are now known in the domestic fowl (Hutt[4], Jull[5], and others). Of these more than half involve the skeleton, while few are known to affect the muscles. However, the domestic fowl does exhibit variation in conformation of the breast and the shape of the breast musculature as shown, for example, by the Cornish as contrasted to most other breeds.

Muscular dystrophies of man are diseases of hereditary bases and are characterized by weakness and altered size of skeletal muscles (Adams, *et al.*[1]). Some of these diseases are characterized by gross enlargement of the involved muscles followed by atrophy. A hereditary muscular abnormality in New Hampshires, with features similar to certain muscular dystrophies of man, is the subject of this report. Genetic aspects are emphasized; however, a preliminary report of observations on the anatomy of the condition is included.

Material and Methods

Eggs from stock known to be heavily muscled on the breast, with some birds unable to raise their wings, were obtained from a New Hampshire breeder in March 1953. Of these, 72 were from a selection designated (by the breeder) Broad Line and 48 were from a Lock Wing selection; 48 of the former and 28 of the latter hatched, and subsequently eight and five respectively (16.7 and 17.9 percent of the birds classified) were found to be unable to raise their wings. The stock constitutes Strain 3 of the New Hampshire breed used by the California Agricultural Experiment Station.

The chicks have been routinely weighed when hatched and at four and eight weeks of age. Some were weighed at 12 and/or 20 weeks and at maturity. The length of the shank (tarso-metatarsus), the keel of the sternum, and the width of the breast 1 cm. above the ventral surface of the keel and near its anterior end were measured at eight or 12 weeks. Some mature birds were also measured. The length of the shank was measured with a special instrument (Burmester and Lerner[3]), the keel with calipers, and the width of breast with soldering wire which was fitted to the breast and then transferred to lined paper.

The birds were classified as normal on the basis of their ability to raise their wings and rise from a flat surface when laid on their backs (Figure 25). Most classifications were made at 8 weeks of age or later. Some were observed to be abnormal at 4 weeks. The age at which the birds show this abnormality varies and may occur for some individuals later than 8 weeks of age. It should also be noted that some Strain 3 birds and birds out of crosses of Strains 3 and 2 have stiff wings that apparently cannot be raised as high as those of Strain 2 birds, yet are "normal" in the sense that they can rise from a flat surface when laid on their backs.

In order to determine how the muscular abnormailty is inherited, the abnormal and normal birds of New Hampshire Strain 3 were mated among themselves and to hens of New Hampshire Strain 2. The latter strain (N.H. Strain 2) has never exhibited muscular symptoms. The wider breasted normal birds of Strain 3 were selected for breeding. The abnormal and Strain 2 birds were not selected except to eliminate sick and obviously unthrifty birds.

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THE EFFECTS OF MUSCULAR DYSTROPHY
Figure 25

Muscular dystrophy prevents this hen from rising from a flat surface when placed on her back.

Several abnormally winged birds kept on the floor fell on their backs and died before they were noticed. The abnormal hens are unable to fly to nests off the ground. Because of these handicaps, abnormal birds kept for breeding were transferred to individual cages before maturity, and all matings involving them have been by artificial insemination.

Birds have been submitted for anatomical study periodically since 1953. In 1955 a systematic study was undertaken in which birds of normal and abnormal stocks were autopsied at 1, 4, 12, and 20 weeks of age. Body measurements and muscle weights were recorded and samples of involved muscles were taken for microscopic study.

Results

Typical hatching results are shown in Table I. Since all the abnormal birds bred to date are out of Strain 3, it is evident that the hatch of fertile eggs from matings of birds within this strain is lower than that of eggs from crosses between strains 3 and 2. This suggests that Strain 3 is inbred and that the improved hatch from crossing reflects hybrid vigor. Were a lethal (or lethals) associated with the muscle abnormality involved, the lowest hatch would have been expected from abnormal birds, which is not the case. Embryos that failed to hatch were examined and no gross morphological deviations that might suggest a lethal were found.

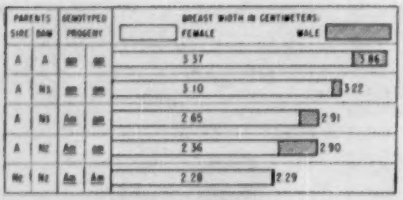
According to limited data, abnormal hens of Strain 3 are slower maturing and lay at a somewhat lower rate than those of Strain 2. Until data are obtained under strictly comparable conditions these data must be considered indicative and not conclusive.

Gain in weight is a little slower for abnormal birds of Strain 3 than for normal birds of Strain 2, but the mature weight is about the same. The abnormal birds have slightly shorter bones, as shown by the shanks (tarsometatarsi) and keels of the sternum, and these differences are found in both young, growing

TABLE I. Hatching results, 1954

Type of mating*		Fertile		Percent of fertile			
				Dead Embryos			
Sire	Dam	Percent	No.	1st week	2nd week	3rd week	Hatched
A	A	78	52	14	4	12	71
A	N3	81	17	6	12	12	71
A	N2	57	90	9	2	6	83
N3	A	85	28	7		25	68
N3	N3	75	66	16	3	16	64
N3	N2	87	41	7	2	5	85

* A is for abnormal, N for normal of strains 3 or 2.



BREAST WIDTH
Figure 26

Effect of the *am* gene on breast width of eight-week-old birds. The width was corrected for differences between average body weights of groups of birds of the same sex. Note that the males were slightly wider breasted than the females.

birds and mature birds. For eight-week-old birds these differences in weight and bone length, while not large, are statistically significant (Table II).

The abnormal birds have much wider breasts, on the average, than normal birds, (Figure 26). Both normal and abnormal birds vary considerably in width (Figure 27), and there is an obvious overlapping of normal birds from Strain 2 on normal from Strain 3 and of progeny between the two strains. Similarly, the narrower breasted abnormal overlap on the wider breasted normal.

Anatomy

A detailed report on the anatomy of the abnormal birds will be presented elsewhere. Preliminary studies indicate that most of the major muscles of the bird are affected by the development of this abnormality. The breast muscles, muscles of all segments of the wings, and muscles of the legs are affected. When enlargement is first detected, the involved muscles are more opaque and somewhat lighter in color than normal. The grossly visible striated nature of skeletal muscle is accentuated in the affected muscles. This is due to enlargement of each fasciculus, which in turn is due to enlargement of each individual muscle fiber. Some of the muscles are more extensively involved than others. As an example, the superficial pectoral muscle (pectoralis major) is more enlarged than its antagonist, the deep pectoral (pectoralis minor); however, gross and microscopic alterations are detected in both. Each muscle apparently has a different pattern of onset and progress of the abnormal state which is superimposed upon its individual normal growth pattern. A major microscopic feature of the enlarged muscles is an increase in number of muscle nuclei, many of which are centrally located in the muscle fibers.

Atrophy appears to be a sequel of muscular enlargement. This phase of the process is variable. Limited data suggest that only enlargement occurs in some animals; atrophic changes, if any, are long delayed. In others atrophy rapidly follows muscular enlargement. Profound microscopic alterations can be demonstrated in the grossly atrophied muscles. These include deposition of fat, degeneration of muscle fibers, and fibrosis. The time of onset of microscopic changes is to be correlated with gross alterations of the muscles. These should result in a fuller appreciation of the full spectrum of manifestations of the condition and should aid in evaluating genetic data.

Although anatomical studies are in preliminary stages, it is evident that the condition of chickens described above has many similarities to the muscular dystrophies of man. The sequence of gross alterations in size of the muscles (hypertrophy and atrophy), the centrally placed nuclei in hypertrophied fibers, and the degenerative changes stated above have their counterparts in muscular diseases of human beings.

Genetics

The data available for various matings are summarized in Table III. Progeny were not obtained from abnormal birds until 1954, partly as noted above, because of the inability of the hens to get into the trapnests and perhaps partly because of their lower egg production.

In 1953-54 mixed semen from several males was used for insemination, whereas the sire and the dam of all birds hatched in 1955 were known. It is not certain that all birds suspected of being abnormal in 1954 were tested by laying them on their backs; however this test was applied to all progeny of abnormal sires mated to abnormal dams in 1955 with the exception of two early dead and 10 birds picked at random for dissection when a week old. A few birds were similarly picked for dissection from other matings in 1955 when they were one week old. Since all chicks that subsequently became abnormal appeared normal when one week old, the birds removed for this dissection are omitted from the table.

The 1953-54 data suggested that abnormal muscling might be a simple autosomal recessive to normal for which the symbol *am* will

TABLE II. Means with standard errors of weights and measurements at eight weeks of age, 1955, of abnormal (Strain 3) and normal (Strain 2) New Hampshires

Classification	Sex	No. of birds	Weight kilos.	Length of shank cm.	Length of keel cm.	Width of breast cm.
Abnormal	male	14	1.02 ± .02	9.17 ± .07	8.11 ± .08	3.67 ± .10
"	female	10	.80 ± .02	8.28 ± .07	7.61 ± .07	3.13 ± .09
Normal	male	123	1.09 ± .01	9.60 ± .04	8.42 ± .04	2.92 ± .04
"	female	109	.86 ± .01	8.68 ± .04	7.63 ± .04	2.67 ± .03

be used. The criterion for abnormality was, as stated above, the inability of the bird to right itself when placed on its back on a flat surface. There is no indication of sex-linkage. Assuming that the difference is determined by a single gene, the 1954 data show these discrepancies: one progeny classified as normal from abnormal parents and three progeny out of normal (N2) dams classified as abnormal when only normal were expected. For these two types of matings (both parents abnormal or one parent out of Strain 2) the 1955 progeny that were classified agree well with expectation. If all the progeny in Table III from segregating families out of normal Strain 3 parents are combined, there were 155 normal to 41 abnormal, which agrees well with expectation of 147 to 49 on the basis of a ratio of 3 : 1. Similarly, the progeny from all segregating families of N3 × A or A × N3 numbered 40 normal to 40 abnormal in agreement with expectation of 1:1, although the 12 progeny from N3 × A parents in 1954 deviate from expectation ($p = .016$). On the other hand, the number of abnormal among the F_2 progeny (original cross, abnormal males mated to Strain 2 females) is less than expected. As noted above, the age at which the birds become abnormal varies and the degree of abnormality may also vary. These variables could well account for the apparent discrepancies in the data. It will, however, be necessary to make further test matings to estimate the effect of gene interaction on the expression of the trait as measured by the ability to raise the wings.

Some of the effects of the *am* gene on conformation and muscles are apparent from the measurements on live birds. Thus, the data in Table II and Figure 26 indicate that the *am* gene influences width of breast, a polygenic trait, mostly determined by additively acting genes and much influenced by body weight[7,6,2]. When the variation normally found in breast width is superimposed on the effect of the *am* gene, the result for older birds is as shown in Figure 27. It will be observed that a few of the abnormal birds were quite wide, thus making this group the homozygous recessives much more variable than the homozygous normal (Strain 2). Detailed anatomical studies now in progress on the 1955 hatch and anticipated for the 1956 hatch of birds may contribute toward a clarification of this situation.

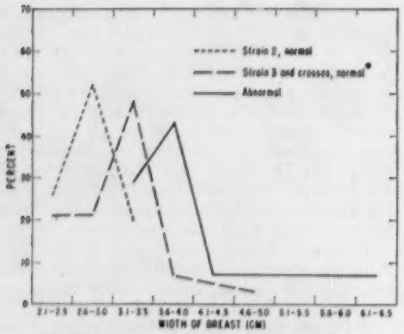
If the *am* gene affects width of breast in the homozygous and heterozygous combinations, the effect being additive so that *am am* > *Am am*, then data for progeny from some of the matings should illustrate this relationship. Figure 26 shows, as does Figure 27, that abnormal birds are wider breasted than normal birds out of the same parents (compare *am am* with *Am am* progeny out of A × N3 parents) and that heterozygous (*Am am*) normal birds are wider breasted than homozygous (*Am Am*) birds. Likewise, backcross progeny from heterozygotes on abnormal or homozygous normal should be more variable, since they are a mixture of two genotypes, than the progeny of the latter, which are all heterozygous. To test this point, in Figure 28 data for the 1955 male progeny of normal Strain 2 females out of abnormal males are compared with progeny of Strain 2 females mated to Strain 3 heterozygous normal males. In agreement with expectation, the latter are more variable;

TABLE III. Summary of results of matings of abnormal and normal muscled birds in 1953, 1954 and 1955. Expected numbers, based on the assumption that abnormal muscling is a simple autosomal recessive, and shown only for segregating families. Progeny classified at eight weeks except as noted in text

Matings*				Progeny†					
				Normal			Abnormal		
Sires	Dams	Year		Males	Females	Total	Males	Females	Total
A	A	1954	Obs.	1		1	9	16	25
"	"	"	Exp.						26
A	N3	1954	Obs.	3	7	10		2	2
"	"	"	Exp.			6			6
A	N2	1954	Obs.	30	40	71	1		1
"	"	"	Exp.			71			0
N3	A	1954	Obs.	3	1	4	1	2	3
"	"	"	Exp.			3.5			3.5
N3	N3	1953-4	Obs.	71	62	133	15	20	35
"	"		Exp.			126			42
N3	N3	" "	Obs.	36	30	66			
N3	N2	1954	Obs.	13	17	30		2	2
"	"	"	Exp.			32			0
A	A	1955	Obs.				19	17	36
A	N3	1955	Obs.	11	12	23	13	12	25
"	"	"	Exp.			24			24
N3	A	1955	Obs.	2	1	3	6	4	10
"	"	"	Exp.			6.5			6.5
N3	N3	1955	Obs.	14	8	22	3	3	6
N3	N3	1955	Exp.			21			7
A	N2	1955	Obs.	22	17	39			
N3	N2	1955	Obs.	30	24	54			
F_1	F_1	1955	Obs.	38	42	80	5	5	10
"	"		Exp.			67.5			22.5

* See footnote Table I.

† Birds that died or were taken for dissection before they could be classified (e.g. when 1 week old) are not included.



ABNORMAL BIRDS ARE WIDER BREASTED
Figure 27

Width of breast in centimeters, one cm. above the ventral surface of the keel of the sternum. Data for 20-week-old males and females combined.

BREAST WIDTH OF MALE PROGENY
Figure 28

Width of breast of eight-week-old birds: broken line—F_2 male progeny of abnormal (*am am*) males and homozygous normal (*Am Am*) Strain N2 females: solid line—male progeny of normal heterozygous (*Am am*) Strain 3 males and Strain N2 females.

about one third are narrower and some wider, than any of the heterozygous progeny of the abnormal × Strain 2 mating. While it presumably would not be possible to segregate homozygous from heterozygous normal on the basis of width of breast, the results illustrated in Figure 28 suggest that the narrowest breasted birds from a backcross population (Figure 28, broken line) should be homozygous normal while the widest breasted are heterozygous. Again it should be emphasized that anatomical data may help to clarify some of the genetic information. As stated above, the superficial pectoral (pectoralis major) muscle exhibits the greatest alteration in size of the muscles that contribute to width of breast. An evaluation of dimensions and masses of individual muscles shows promise of contributing toward a solution of these problems.

The simplest explanation for the data so far available is that abnormal muscling, as shown by the inability of the birds to raise their wings, is a simple autosomal recessive and behaves as such within Strain 3 of the New Hampshire breed. The possibility exists that genes carried by other strains and breeds may modify the age at which this trait appears and its severity of expression to a greater extent than those in Strain 3. The gene also affects width of breast and other traits, but here its effect is modified, though not obliterated, by many other genes. Further work on the genetics of the trait is under way.

Summary

A muscle abnormality, which prevents the birds from raising their wings and rising from a flat surface when laid on their backs, has occurred in New Hampshires. When compared with normal birds of this breed but of a different strain (Strain No. 2), in which muscular symptoms have not occurred, the abnormal birds were found to be wider breasted and to have shorter bones.

Preliminary anatomical studies show that most of the muscles of the bird are involved. Gross and microscopic alterations of the muscles are comparable to recorded changes in muscular dystrophies of man.

The data, from various types of matings, indicate that abnormal birds are homozygous for an autosomal gene (*am*) which is recessive to normal.

Heterozygous (normal) birds have wider breasts than homozygous normal birds of the New Hampshire breed.

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